PARASITOLOGY

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PARASITOLOGY

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THE POST-EMBRYONIC DEVELOPMENT OF *PHAENOSERPHUS VIATOR* HAL. (PROCTOTRYPOIDEA), A PARASITE OF THE LARVA OF *PTEROSTICHUS NIGER* (CARABIDAE), WITH NOTES ON THE ANATOMY OF THE LARVA.

BY L. E. S. EASTHAM.

(*Department of Zoology, Cambridge.*)

(With Plates I–III and 1 Text-figure.)

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I. INTRODUCTION AND HISTORICAL.

THE family Proctotrypidae would seem to be one of the most neglected of the Hymenoptera parasitica although, so far as is known, its members are entirely insect parasites and therefore of great economic importance.

Marchal (1906), Ayers (1884), Haviland (1920), and Chophard (1922), among others, have contributed largely to our knowledge on nearly related families and, apart from the systematic works of Ashmead (1893), Morley (1922), Kieffer (1914), and André (1879), there seems to be little in the literature except short natural history notes which possess some value, in that they indicate a field of entomology still to be explored.

This paper gives a short review of the Proctotrypid parasites and their specific hosts, in the hope that it may be useful to those whose work lies in this direction.

According to Comstock (1925), and Imms (1925), members of the superfamily Proctotrypoidea are largely parasitic on the larvae of Coleoptera, Diptera, Orthoptera, Hemiptera and Lepidoptera. In 1923 Bischoff reported larval hosts among the Aphidae, Cecidomyidae, Mycetophilidae, Muscidae, Syrphidae, Scolytidae, Staphylinidae, Curculionidae, Coccinellidae, Tineidae, Tortricidae, Neuroptera Planipennia, Formicidae, and Myriapoda. The eggs of Lepidoptera, Rhynchota, Neuroptera Planipennia, Trichoptera, Odonata and Coleoptera are also parasitised by them. Morley (1922) finds that in addition to the above, the Carabidae and Elateridae suffer from their attentions.

The cosmopolitan tastes indicated above may be taken as an index of the importance of the Proctotrypoidea in the realm of economic entomology.

The family Proctotrypidae is however more restricted than the superfamily, its hosts being chiefly found among the orders Diptera and Coleoptera. The first reference to a parasitic association between a Proctotrypid and a beetle is that quoted (from the *Boston Journ. Nat. Hist.* 1836) by Elliott and Morley (1911), *Proctotrupes obsoletus*, say, being bred from *Stelidota strigosa*. In 1855 Kawall bred *Codrus pallipes* from a dead Staphylinid, and in 1857 Kirby reports *Proctotrupes* sp. as parasitic on larvae of *Agriotes*. Curtis bred *Proctotrupes viator* (?) from *Nebria brevicollis* in 1883, and also reports an unknown Proctotrypid parasitic on *Agriotes obscurus*. Newman (1867) bred *P. calcar* from *Lithobius* sp. In 1868 Smith found a larva of *Xanthalmus* with attached Proctotrypids. Frohawk in 1886 gave a short account of *Proctotrupes ater*, of which there were nine pupae, attached to the ventral side of a larva of *Ocypus olens*.

Lists of Hymenopterous parasites of Coleoptera were drawn up in 1907 and 1911 by Elliott and Morley; and of the Proctotrypidae they mention the following species of *Proctotrupes:*

P. viator	bred from	*Pterostichus vulgaris*,
P. viator	„	„	*Nebria brevicollis*,
P. pallidipes	„	„	*Ocypus olens*,
P. sp.	„	„	*Agriotes obscurus*,
P. sp.	„	„	*A. lineatus*,
P. ater	„	„	*Ocypus olens*,
P. obsoletus	„	„	*Stelidota strigosa*,
P. calcar	„	„	the Centipede *Lithobius* sp.

Bischoff (1923) adds the following references to those of Elliott and Morley (1907, 1911).

Proctotrupes (various unnamed) were bred from Mycetophilid larvae,
Serphus divagator was bred from *Lonchaea* (Diptera),
Exallonyx subceratus was bred from pupae of *Phora rufipes* (Diptera),
Serphus reicherti was bred from larvae of *Quedius* (Coleoptera),
Exallonyx lagatus was bred from larvae of *Quedius* (Coleoptera).

Morley (1922) adds to his previous lists the following:

Proctotrupes aculeator was bred from *Mycetophila nigra*,
P. parvulus was bred from the fungus *Boletus*, possibly having been parasitic on the beetles *Orchesia micans* and *Palacrus corruscus*, or hyperparasitic on the Ichneumon *Thersilochus orchesiae*,
P. gravidator was bred from *Cynips kollari* and *Boletophila fusca* (Diptera),
P. devagator was bred from *Sciophila linn* (Diptera),
P. pallipes was bred from *Macrocera maculata* (Diptera),
P. niger was bred from *Brachycampta griseicollis* (Diptera),
P. ater was bred from *Mycetophila punctata* (Diptera),
P. apterogynus was bred from *Agriotes* sp.

Zolk (1924) gives a short account of *Paracodrus* (*Proctotrupes*) *apterogynus* which parasitises the larva of *Agriotes obscurus*. This is the most important account so far published, last larval and pupal stages being described.

Except for the latter case cited, all the above references are to chance discoveries during the pursuance of other work, little more than a mere statement of the parasitism being made. It was therefore deemed advisable to continue my observations on as much material as could be collected. Material was never obtained in large quantity and several years passed before sufficient publishable information was obtained.

During the course of this investigation, larvae of *Pterostichus niger* were taken from many districts in Warwickshire, Worcestershire, Staffordshire, and Lancashire, and more latterly Cambridgeshire. They were examined for parasites by dissection in normal salt solution. The parasites found were examined alive as far as possible, then fixed in alcoholic bouin or Carnoy's fixative for further reference. Whole mounts were stained in borax carmine or Delafield's haematoxylin. Sections were cut on the Cambridge rocking microtome and stained with Heidenhain's haematoxylin, using eosin as a counter stain.

Any *Pterostichus* larvae found in a district where infection occurred were kept separately in boxes of soil in the dark, and from such stock specimens were examined at fortnightly intervals till the time of emergence of the parasites. All attempts to infect host larvae failed.

I take this opportunity of thanking Mr W. Leach of Birmingham University for providing me with the first specimen of a parasitised beetle larva. To Miss E. Sikes, formerly of Birmingham University, I am indebted for supplying me with material on many occasions, and to Dr J. Waterston for identifying the parasites in the adult stage.

II. Life-history.

Phaenoserphus viator, according to André (1879), has a wide range in Southern Europe from the Crimea to France. Morley (1922) reports it as being common in England and Ireland. Although *Phaenoserphus* was never observed to oviposit in the larva of *Pterostichus niger*, measurements (see p. 6) of the larval instars clearly indicate that the life-history is annual.

Adults emerge in August and September and can be found visiting flowers or searching the upper layers of the soil. From September onwards, dissections of *Pterostichus* larvae showed that larval life lasts till the middle of August. Then follows a prepupal stage of about a week or 10 days, followed by a pupal stage of about 2 weeks' duration. From 9 to 45 larvae were found in the haemocael of a single host. During the winter months, October–March, there is little growth made by the parasites. This was first assumed to be due to hibernation of both host and parasites. It must be noted, however, that Pantel (1909) stressed the asthenia exhibited by parasites in their earliest stages, and Roubaud (1922) attributes this to the parasitic association in which the hosts' blood is sufficiently toxic, by reason of its contained excretory matter, to keep the early stages of the parasite in a state of asphyxia. During April the host larvae lead an active life, and it is now that the contained parasites begin to grow rapidly.

During May the host larva becomes sluggish and at the end of this month and the beginning of June, *Pterostichus* larvae which are infected will commonly be found lying in soil cells, stretched out unable to walk, as if preparing for pupation. They respond to touch as found by Zolk (1924), but by the end of June even this sign of life vanishes. From now onwards the host larva shows distinct indications of its contained parasites. Its body becomes stretched out tight, all arthrodial membranes of limbs and segments being extended, and as the parasites grow bigger they raise the skin of the host into more or less parallel ridges. It will be seen therefore that during May–August there is no difficulty in determining whether a larva is parasitised or not. The parasites finally ramify into all parts of the host, into the head capsule and into the hollow limbs. When fully grown in August, and when there is no more host tissue left as food, the parasites emerge through the arthrodial membranes between the sternites of the thorax and abdomen. The parasites may be seen to move within the host's skin before emergence. Their final arrangement is nearly regularly longitudinal and with heads pointing towards the posterior end of the host larva. The larvae emerge intersegmentally in rows of three or four. They protrude from the host, exposing head, thorax and half of the abdomen and at the end of the emergence as many as thirty or more parasites will be seen protruding from the ventral surface of the host, each with its ventral surface pointing to the head of the host. Such definite orientation of parasite to host is obviously the result of the position taken up by the larvae within the host prior to emergence. It occurs with remarkable regularity and has been noted by Sharp (1910), and Frohawk (1886). The parasites remain in this position till the adults emerge, and spin no cocoons before pupation. The larvae cast their thin transparent cuticles as soon as they emerge and a long prepupal instar now sets in, which under laboratory conditions lasts for a week or 10 days, during which the imaginal appendages gradually become everted so as to be easily visible through the cuticle. Larvae, prepupae and pupae are white on their first appearance. About a week after the beginning

of the pupal stage, the ocelli and eyes begin to darken and in the next 2 days the whole body becomes nearly black, the colour of the adult. From 5 to 7 days later the adults emerge leaving behind them the transparent colourless pupal exuviae. Text-fig. 1, C shows the pupae in characteristic position attached to the host; a similar relation is found in last larval and prepupal instars. The flies remain round the host for about 24 hours, then proceed to search through the upper layers of soil presumably for other host larvae to infect.

An interesting feature in this life-history is the effect of the parasite on the metamorphosis of the host. The life-history of *Pterostichus niger*, given by Blunck (1925), is as follows. The egg is laid in June. The larva hatches in July and continues through several instars till May of the next year, when it pupates in a mud case in the soil. In collecting the larvae of *Pterostichus*, it was observed that pupation occurred from the middle of April till the middle of May. In the first instance it is noticed that the parasitised larvae fail to pupate; secondly, the maximum growth of the parasites is sure to take place from the time when pupation would normally occur.

The growth of the parasites may be due to the host tissues having been partly phagocytosed and therefore rendered more accessible to the parasites, but no evidence was found to support this view, the host tissues remaining always in the larval state. Pantel (1909) finds that in the early stages of a parasitic association between two insects the parasites grow slowly. Roubaud (1922) attributes this to the toxic effect of the host fluids on the parasites. After a period of slow growth on the part of the latter, the relation between parasite and host becomes reversed, the parasites gaining the ascendancy. Inhibition of host metamorphosis caused by the parasites might well be an expression of this dominance. The more direct effect of starvation produced by the parasites must not be overlooked. Consumption of larval fat-body and body fluid would obviously prevent the host from storing up reserve material necessary for pupation. The effect of starvation so produced would confirm Singh-Pruthi's conclusions (1925) on the effect of starvation on the metamorphosis of *Tenebrio molitor*, metamorphosis being retarded by starvation in the case of immature larvae. That parasites do have a clearly marked effect on the metamorphosis of the host is also shown by the work of Pemberton and Willard (1918). Working at *Diachasma tryoni*, a Braconid parasite of the pupa of *Cerabitis capitata* (Diptera), they found that the mere insertion of an egg into a pupa inhibited histogenic changes. If the egg were laid in the host larva, the latter went on feeding; histolysis occurred at the beginning of pupation, but not histogenesis. This is all the more remarkable when we consider Perez's work on the metamorphosis of the blow-fly (1910) which shows that at pupation histogenesis and histolysis set in concurrently. It may easily be that a study of the effect of parasite on host in insects may open a new method of attack on that difficult subject, viz. the conditions which govern the onset of histolytic and histogenic changes in insect metamorphosis.

III. Larval forms.

As is the case with all endoparasitic insects, examination of the parasites at any stage of development involves the death of the host. The full development of any individual parasite cannot be observed. The uncertainty of investigation, arising from this, can however be obviated by the examination of specimens at short intervals throughout several life-cycle periods. It is for this reason that the research into the life-history of *Phaenoserphus viator* has been carried on through several seasons. In these seasons the stages found corresponded month for month, as they were found in the successive years. Without such agreement there would be no certainty that the same parasite was being dealt with throughout. The impossibility of following the full story of a single parasite renders the determination of the number of instars uncertain. The progressive differentiation of the tracheal system as determined by Haviland (1920) on *Lygocerus testaceimanus* gives a good indication of the number of instars. In the case of *Phaenoserphus*, four larval instars are indicated. Table I records the larval measurements and shows that the greatest growth takes place from the beginning of May, little or no growth occurring in winter.

Table I.

Date of Examination	Length in mm.	Greatest width in mm.	Probable instars
Oct. 15	0·75	0·153	1st
Dec. 12[1]	0·84	0·165	1st
April 10	0·83	0·163	1st
May 12	1·5	0·3	1st or 2nd
May 19	1·67	0·3	2nd
June 1	2·13	0·37	2nd or 3rd
June 16	2·8	0·65	3rd
July 3	3·8	0·8	4th
July 31	4·4	1·1	4th
Aug. 10	4·6	1·1	4th

[1] All measurements taken during winter are not included, there being no obvious increase in length.

(1) *The first observed larva.*

During the months October to May, in which the first observed larval form was found, the measurements and comparative study of mouth-parts and prolegs clearly indicate that only one instar occurs. The host larva hibernates till March or April and the parasite shares its inactivity, undergoing no increase in size (see Table I). The first observed lava is caudate (Pl. I, fig. 1). It is comparatively active, bending on itself by turning its caudal extremity up and down; its mandibles meanwhile operating rapidly in a pincer-like manner in front of the mouth. Nothing but fluid seems to be absorbed. The mid-gut and its contents are transparent and present a very different appearance to what is seen in the next instars, when the mid-gut is opaque and full of more viscous material in which particles of fat can be seen. The larva consists of

a head and ten post-cephalic segments. Of these, the last is by far the largest, and is in fact a macro-segment from which four segments will ultimately differentiate. The body as a whole is curved ventrally, with a rounded head attached without prominent constriction to the next segment. The third and fourth post-cephalic segments are the broadest, and from here the body tapers towards the caudal extremity which is bluntly pointed.

The Head. Although the larva at this stage is incompletely developed posteriorly, the head bears its full complement of appendages though in a simplified state (Pl. I, fig. 2). A pair of antennary papillae are found on the anterior convex surface of the head. On the ventral surface of the head is situated the transverse opening of the mouth, its anterior border formed by the labrum, its posterior by the labium. The labrum is a simple overhanging lobe to the mouth and bears chitinous teeth on its under surface. The chitinous mandibles lie on each side of the mouth. Each is sickle-shaped with a broad base bearing inner and outer condyles for the attachment of its appropriate muscles. When at rest the mandibles lie opposed to one another over the mouth, with their convex border in front. They have great freedom of movement in the horizontal plane and when fully extended project forwards, parallel to one another, beyond the anterior surface of the head. Behind and mesad to the base of each mandible is inserted the base of the maxilla. The maxillae are cylindrical fleshy lobes slightly tapering to a blunt apex. Each bears about three small papillae at or near its tip. The labium forms a soft lower lip to the mouth and the only sign of its bifid constitution lies in the two blunt prominences on its edge, which are only discernible under very favourable conditions of examination. Although only slightly chitinous externally, its inner surface forming the floor of the buccal cavity is well lined with chitin. The mouth parts and buccal cavity are supported by a chitinous ring which represents the rudiment of a head capsule. This cephalic ring projects slightly in front in the region of the antennary papillae. Laterally the ring bears blunt prominences on its inner surface, and into the angles of these fit the bases of the mandibles. Posteriorly and ventrally the ring is completed in the body of the labium. A broad plate-like extension passes backward from the anterior part of the cephalic ring above the buccal cavity. Posterior extensions from this give attachment to head muscles.

The segments behind the head are simple in nature and are remarkable at this stage in that some of them possess paired appendages (Pl. I, figs. 1 and 3). These occur on post-cephalic segments 2–8. Each such proleg is a blunt conical protuberance attached ventro-laterally, and showing a division into three segments, the terminal one being the smallest and often invaginated into its more proximal neighbour (Pl. I, fig. 4). Slight prominences were sometimes found in the first as well as the ninth post-cephalic segment but their occurrence was inconstant. In this stage no anus appears to be present, though the hind-gut can be seen to extend through the greater part of the unsegmented region. Towards the latter end of the winter months segmentation

takes place in the posterior region (Pl. I, fig. 3), so that the abdomen is now completely segmented. The terminal segment is clearly defined from the rest, possessing a vertical fin-like projection of chitin both dorsally and ventrally. The anal aperture now present is dorsal, and the hypodermal end of the hind-gut makes connection with the exterior by a fine canal which perforates the dorsal chitinous fin. The last segment projecting beyond the anus retains the caudal feature of the larva. At the first appearance of posterior segmentation, the larva measures about 0·8 mm. in length. In May I was fortunate enough to dissect a host in which the parasites were on the point of ecdysis. There were no fewer than 40 parasites, each of which measured approximately 1·5 mm. in length. These larvae were of the type just described agreeing in all details except in point of size. The parasites moulted, cast off the prolegs, legless larvae resulting.

Caudate larvae have been observed in first instars in other parasitic Hymenoptera. The most striking examples are found in the Ichneumonoidea, where the last segment of the body is extended backwards beyond the anus to form a tail, e.g. in the Braconid *Mesochorus*, Seurat (1899). In *Microgaster* and *Apanteles* the tail is in the form of a vesicle, which Gatenby (1919) in *Microgaster* has shown consists of a proctodæal evagination.

In *Phaenoserphus viator* the tail is formed primarily of a macro-segment from which four true segments are later defined, so reducing the caudal appendage to a short extension of the last body segment as in the Braconid *Mesochorus*, Seurat (1899). Incomplete segmentation of the abdomen has been observed in the primary larvae of *Synopeas rhanis* by Marchal (1906), in the Proctotrypid *Teleas* by Ayers (1884), in the Proctotrypid *Rielia manticiola* by Chophard (1922), by Clausen (1923) in the Chalcid *Schizaspidia venuicornis*, by Parker (1924) in the Chalcid *Psilogaster furciiventris*, and by Keilin and Pluvinel (1913) in the Cynipid *Eucoila keilini*.

The fact that this is a feature found in certain Chalcids, Cynipids and Proctotrypids alike, renders the possibility of its being of any phylogenetic significance very remote. It must rather be referred to conditions of development in which amount of yolk, nature of the host, position of the egg in the host might easily be regarded as factors affecting development in such a way as to cause more rapid differentiation anteriorly than posteriorly. In general, parasitic insect larvae of the form referred to have been regarded as embryonic in nature. Thus Marchal (1906) regards the Cyclopid larva of *Trichacis* as analogous to the crustacean Nauplius and its appearance due to a small amount of yolk in the egg. The embryo therefore hatches earlier and appears in a form which is normally passed through in the embryo. Keilin and Pluvinel (1913), in discussing larval forms, also conclude that paucity of yolk accounts for the precocious hatching of the larva. While admitting that a small amount of yolk contributes to the production of so-called "embryonic" larval forms, it seems reasonable to assume that all other factors controlling growth should be taken account of. Such factors may be grouped together as the environment

in which the egg finds itself during development. There appears as yet to be little information on the relative amounts of yolk found in the eggs of parasitic insects, and till that is known dogmatic statements about the effect of paucity of yolk on hatching are scarcely warranted.

The first observed larva of *Phaenoserphus buccatus* calls for further remarks in connection with the rudimentary appendages found on the thorax and abdomen. Berlese (1913) divides the embryonic development of insects into four stages: (1) Protopod oligomero, in which the abdomen is unsegmented; (2) Protopod polymero, in which the abdomen is segmented; (3) Polypod, in which the segmented abdomen bears paired rudimentary appendages, and (4) Oligopod, in which these latter are lost, the thorax and head alone retaining appendages (see also Imms, 1925). Normally it is in the last stage that the insect hatches from the egg. On the assumption that certain parasitic larvae hatch precociously in an embryonic condition, such larvae are compared with one or other of Berlese's stages, see James (1928). In the case of *Phaenoserphus viator* it is difficult to assign the first observed larva to any one of Berlese's stages. Posteriorly it corresponds to Berlese's Protopod oligomero stage in that the last four body segments are not defined. In the anterior abdominal region it corresponds with Berlese's Polypod stage, while the head is so well developed as to be called larval in the true sense of the word and cannot with justice be compared with any embryonic stage at all.

The posterior region of the body, in passing from an unsegmented to a segmented state with transient rudimentary appendages, conforms quite well however with Berlese's order of development as in fact will any other isolated region of the body. The difficulty lies in comparing the whole larva at any one stage in its life with any single embryonic state.

The appendages formed on the body of *Phaenoserphus viator* may be either (*a*) ancestral (this may be assumed by homologising the primary larva with Berlese's embryonic stages) or (*b*) purely adaptive.

In the case of the Cynipid *Eucoila keilini*, Keilin et Pluvinel (1913), the appendages are regarded by these authors as having no ancestral significance, being purely adaptive and used as organs of locomotion.

In *Phaenoserphus* movements are executed by flexure of the body and the appendages play no part in locomotion, and, unless they have some other function, *e.g.* respiratory, it is difficult to regard them as anything but vestiges.

The polypod larva recalls similar larval forms described by James (1928) in certain Cynipids. Reference to his account renders it probable that a primary larva may exist before that already described in *Phaenoserphus*.

(2) *Subsequent larval instars.*

Subsequent larval stages differ from the one described in size and in the matter of mouth-parts, body appendages, and in the position of the anus.

Since moulting is seldom observed in parasitic larvae, some other method

has to be employed for distinguishing between the separate instars. The method of Haviland (1920), that of noting important changes in tracheal development from the first to last larval stages, has been adopted.

In *Phaenoserphus*, although the first observed larva is atracheate, future spiracles are indicated by paired slight thickenings of hypodermis in the meso- and meta-thoracic and the first six abdominal segments. In larvae found in May, possessing no paired appendages and measuring about 1·5 mm. in length, these hypodermal thickenings have developed into invaginations with forward and backward extensions which have joined to form a pair of short longitudinal non-functional tracheae in the thorax. In the abdomen however the spiracular twigs have not joined up to form longitudinal tracheae. This development indicates a second instar, a fact proved by observing ecdysis (see p. 8).

A third instar is indicated in larvae found early in June and measuring about 2·5 mm. in length. Here the thoracic and six abdominal spiracles are connected to a pair of longitudinal tracheae passing down the sides of the body. Further, in this stage, short dorsal and ventral twigs have developed from the longitudinal trunks, but have not yet attained contact with organs. Spiracular thickenings, developing rapidly into invaginations, are also found now on abdominal segments 7 and 8. The tracheae contain no air, and no chitinous lining is yet present.

The fourth and last instar is of long duration, and growth from about 3 mm. to 4·8 mm. in length is effected. Here two thoracic and eight abdominal spiracles are present. Air is contained in the tracheae and the now open spiracles are visible on the surface of the body as minute brown spots, the thoracic and first abdominal spiracles being largest. The difference in size, arising from their order of development from before backwards, is possibly an adaptation to the habit of emergence, in which the greater part of the abdomen often remains embedded in the skin of the host larva, the posterior abdominal spiracles being thereby rendered of less importance than the anterior.

Returning to the examination of the external morphology of the second, third and fourth instars in question, the second instar larva measures about 1·5 mm. to 2·0 mm. in length, its greatest diameter being 0·3 mm. It possesses no paired appendages on the body and, its anus being terminal, the larva can no longer be described as caudate (Pl. I, fig. 7). Its mouth-parts resemble those of the first stage larva. The third and fourth instars differ from the last only in mouth-part differentiation. In these instars the labrum projects over the mouth as a rounded beak, and in consequence the transverse opening of the mouth appears to be turned abruptly forwards at the sides (Pl. I, fig. 5). Both borders of the mouth are chitinous as also are the mandibles. The latter are shorter than in earlier instars; they consist of broad chitinous non-dentate plates, ending in curved points which turn inwards but fail to meet over the mouth; they lie along the outer posterior borders of the mouth, and can only serve a small part in feeding. The maxillae are a pair of blunt lobes found immediately behind the mandibles, their apices lying opposite the median

posterior angle of the mouth. The labium, which forms the posterior border to the mouth, bears the salivary duct in its median portion, which is slightly marked off from small lateral lobes that lie close to the maxillae at their sides.

From the disposition and nature of the mouth-parts described it will be seen that feeding is now largely suctorial, the mandibles having become vestigial. This is confirmed by observation on the living larva when the labrum and labium are seen to work actively over the mouth against the soft tissues of the host, the mandibles and maxillae being motionless.

At the end of larval life the shape of the mouth alters rapidly from that described, to an almost straight transverse slit in the prepupa (Pl. I, fig. 6). Consequently the mandibles and maxillae are carried backwards right away from the mouth, and lie on the postoral surface of the head. The mouth-parts of the closely allied species *Paracodrus apterogynus*, as figured by Zolk (1924), are very similar to those of *Phaenoserphus*. His interpretation of them is evidently very different from mine of the mouth-parts of *Phaenoserphus*. The small lateral lobes joined across the posterior border of the mouth, the labium of *Phaenoserphus*, he calls the mandibles. The next pair which I designate mandibles he calls maxillae and for labium he has a transverse band having no relation to the mouth. A pair of lobes, lying behind the organs which he calls maxillae, is ignored by him. These obviously constitute the real maxillae.

A study of the mouth-parts of the prepupa is most illuminating in this connection; their true nature is revealed by their imaginal discs which can be seen through the skin (Pl. I, fig. 6). The most anterior discs lie within the chitinous mandibles, the others are in relation with the maxillae and labium which appear in order behind them.

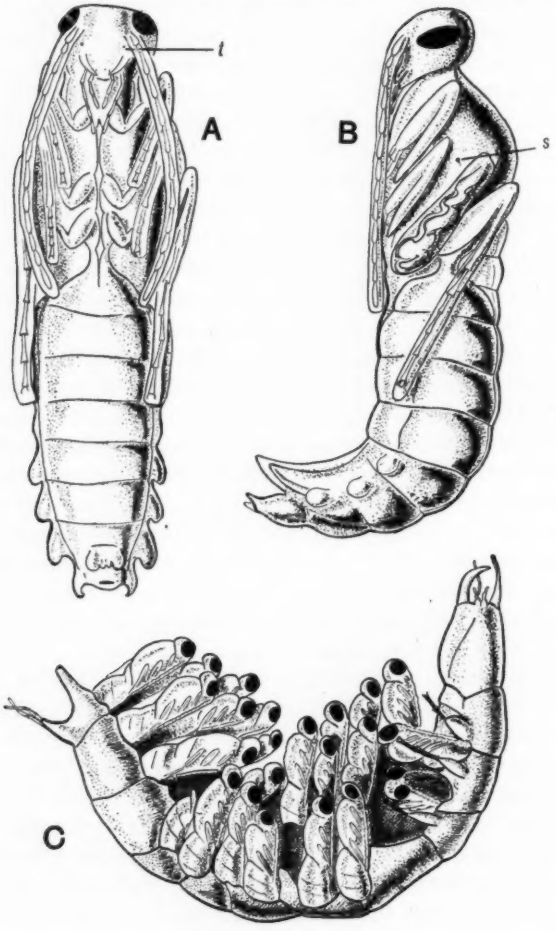
The tripartite imaginal discs of the labium lie in the median line between the maxillae and are removed a little distance behind the labium. The development of imaginal discs for the several mouth-parts can be traced through the prepupal stage till they finally attain the adult form. Such observations remove any doubt as to the homology of the appendages of the larva, which might otherwise be difficult to interpret. It might be added that it is difficult to imagine the undoubted mandible of the early larva being converted into a mere posterior lobe of the mouth, and the fleshy maxillary lobe of the early larva being converted into the mandible-like organ of the late larva, as must be assumed if Zolk's interpretation is right.

IV. The pupa (Text-fig. 1, A and B).

The pupa is white on its first appearance but from the seventh day onwards it steadily darkens till the whole body is nearly black. It measures 4·7 × 1·1 mm., the petiole marking the middle point of its length; the proportional measurements of the head, thorax, and abdomen being respectively 1 : 4 : 5.

Text-fig. 1, A and B, clearly indicates the morphological features of both male and female pupae and will serve instead of a lengthy description. The presence of paired hooked lobes on the last four segments of the abdomen is

worthy of note, and, since these segments during pupation are embedded in the cuticle of the host, they may be regarded as organs of attachment.



Text-fig. 1. A. *Phaenoserphus viator*, ♂ pupa, ventral view.
B. *P. viator*, ♀ pupa, lateral view.
C. *P. viator*, pupae protruding from dead larva of *Pterostichus niger*.

V. Anatomy of the larva.

(1) *Alimentary canal* (Pl. II, figs. 8, 9 and 10).

The alimentary canal is straight, its three portions, fore-, mid- and hind-gut, being well defined. The fore-gut, comprising buccal cavity and oesophagus, extends to the prothoracic segment, the junction of mid- and hind-gut being found about the level of the eighth abdominal segment. The buccal cavity is

well chitinised and its roof, continuous with the body of the labrum, gives attachment to the labral muscles. These, clearly indicated in Pl. II, fig. 8, are dorso-ventrals, transverse, and longitudinals. The dorso-ventrals occur only in the median sagittal plane. The longitudinal muscles pass from before backwards from the tip of the labrum to the beginning of the oesophagus, the transverse muscles interdigitating with them. The oesophagus is short and passes through the brain mass. It is lined with chitin and consists of a columnar epithelium provided with an inner layer of circular muscles. The whole is enclosed in a connective tissue sheath which also envelopes the aorta.

Oesophageal and mid-gut elements meet to form the oesophageal valve. Pl. II, fig. 9 indicates the extent to which these elements have become modified in forming the valve. A striking feature is that oesophageal cells become piled up near the inner end of the valve so as nearly to obliterate the lumen at that point. These cells, which show signs of disintegration, become liberated into the mesenteron cavity and mingle with the food mass. They are probably digestive, and not concerned with peritrophic membrane formation as described by Richardson (1913).

The mid-gut is a wide sac lined by a cubical epithelium in which replacement cells are almost absent (Pl. II, fig. 10). The epithelial surface is smooth, no striated border being present. The musculature of the mesenteron is very delicate, and consists of a fine net-work of transverse and longitudinal fibres. A strong peritrophic sac (Pl. II, figs. 9, 11 and Pl. III, fig. 12) is present in the mid-gut, which hangs freely into the lumen from its point of attachment at the junction of oesophagus and mesenteron in the valve. This point is probably the place where the peritrophic membrane originates. It shows no sign of being laid down in layers as described in *Apis* by Pavlovsky and Zarin (1922). Pl. III, fig. 12 represents the junction of mid- and hind-gut. It will be seen that mesenteron and hind-gut elements share in the closure at this point. The hind-gut (Pl. III, fig. 12) possesses a strong musculature of longitudinal and circular fibres throughout. It comprises three sections, (*a*) a "closure" portion already mentioned, (*b*) the colon, which is distinct from the next section by reason of its shorter epithelium and narrower lumen, (*c*) the rectum, whose lumen is wide, bounded by tall columnar cells. At the beginning of the colon a ring of cells is found at the base of the epithelium; these cells are imaginal elements for renovation of the proctodaeum at metamorphosis.

(2) *Accessory organs to the Alimentary Canal.*

Salivary glands. These are simple tubular organs and well developed in all stages. They lie ventro-laterally to the alimentary canal and unite to form a common duct at the level of the prothorax. The common duct passes forward below the nerve cord and opens in the middle of the lip of the labium.

Each gland has a chitinous lining surrounded by very large irregular-shaped cells (Pl. II, fig. 10). In the mature larva the gland measures 90μ in

diameter. In early stages it is to be noted that the cells of the two glands make connections with one another at the posterior ends of the glands.

Malpighian tubules (Pl. II, fig. 10). There are three malpighian tubules which pass into the gut laterally just behind the mid-gut closure. A short single one on the left side passes backwards. The other two pass forwards, one on each side of the gut, and originate as a short common trunk emerging from the junction of mid- and hind-gut, on the right side.

(3) *Tracheal system.*

There are ten pairs of spiracles in the mature larva, found in all segments from the mesothorax to the eighth abdominal segment (Pl. I, fig. 7). From these, spiracular twigs pass inwards to a pair of lateral longitudinal trunks connected with one another by a dorsal commissure in the prothorax. No posterior commissure exists, as found by Seurat (1899) to be present in Ichneumonids and Chalcids, by Imms (1916) in *Aphelinus*, by Haviland (1920) in *Lygocerus* and Parker (1924) in Chalcids. The longitudinal tracheae end anteriorly in a set of branches supplying the head, and posteriorly in fine twigs which supply the segments behind the last pair of spiracles.

From the junction of the spiracular branch and the longitudinal tracheae, dorsal and ventral branches are given off which aerate the body wall and viscera.

In the development of the tracheal system the last stage is that in which tracheole cells extend outwards from the tracheae to the various organs. Such cells are much attenuated, possess oval nuclei, and within their cytoplasm there develop intracellular canals communicating with the lumen of the tracheae from which they arise (Pl. III, figs. 13 and 14).

It is interesting that these cells have only been found at the beginning of the last larval instar, and this, coupled with the absence of gas in the tracheal system till the final instar, raises the interesting question of how gas enters a tracheal system not in communication with the atmosphere. Weismann (1863) was the first to offer any suggestion on this matter, saying that there must occur a resorption of fluid in the ultimate points of the tracheal system to render possible the entrance of gas. He also inferred that the beginning of movement on the part of the larva will cause pressure to be brought to bear on the main stem so that air is pressed into the fine ends of the tracheal system. It was not till 1924 that this matter was clarified by definite observation.

Keilin (1924) made interesting observations on the subject of the first appearance of gas in tracheae in the larva of *Dashyhelea obscura*. He observed that gas occurs first in the main tracheae and later in the capillaries, and concluded that entrance of gas and removal of tracheal fluid take place simultaneously. As the tracheal fluid is absorbed from the intra-cellular capillary tracheoles by the surrounding tissues, the column of fluid is ruptured and the space left is filled with gas which diffuses in from surrounding tissues.

In Keilin's larva, presumably, the whole tracheal system is developed when the animal hatches. In *Phaenoserphus* however development is more gradual,

taking place progressively through several instars. It is only in fourth larval instars that gas is seen in the tracheal system, *i.e.* after the end cells of the tracheal system have grown out and become connected to the surrounding tissues. Till such connection is effected no absorption of tracheal fluid can take place and no gas enter the trachea in its place. Keilin's observations, therefore, are confirmed by mine on the tracheal development of *Phaenoserphus*.

(4) *Circulatory system* (Pl. III, figs. 15 and 16).

The dorsal vessel extends from the posterior end of the abdomen to the head. Its abdominal portion may be called the heart, being contractile and carrying paired ostia, the thoracic and cephalic parts comprising the aorta, which is conductive but not propulsive. It is blind behind, possesses five pairs of lateral ostia in the first five abdominal segments and then rapidly becomes narrow, its lumen diminishing in diameter from 50μ to 20μ.

Throughout the whole of the abdomen and the metathorax it is closely connected to the dorsal hypodermis, the hypodermis being thickened and slightly invaginated along the line of attachment (Pl. II, fig. 11). Two layers of cells are clearly visible, (*a*) an outer layer of longitudinally arranged anastomosing cells obviously comparable to the adventitious layer of Korschelt (1923), and (*b*) within this, a delicate layer of cells arranged in a circular manner and more strongly developed in the valvular regions. I can detect no striations, but from the general arrangement they must be muscle-cells, whose transverse striations are not yet differentiated.

The internal layer (*b*) becomes developed most strongly behind each pair of ostia, and projects forward into the heart tube on each side to form a pair of ventricular valves which are attached dorsally and ventrally to the heart wall. Pl. III, fig. 16 shows a sagittal section of the heart, in which the inner circular cells forming the valve of one side is included. The valves so formed act as auricular as well as ventricular valves, no special ventricular valves being differentiated as in the Odonata (Tillyard, 1917).

The heart rests on a fine muscular pericardial diaphragm (Pl. III, fig. 15), consisting of elongated muscle cells passing from the outer wall of the heart in its ventral portion to the lateral body wall. Certain fibres pass outwards from the lateral wall of the heart to the pericardial diaphragm, and in the space enclosed between these fibres and the main diaphragm, are to be found the pericardial nephrocytes. In the abdominal region a mesentery of cells hangs downwards from the ventral wall of the heart. This curtain projects freely into the body cavity, and probably consists of additional pericardial nephrocytes (Pl. III, fig. 15). Many muscle fibres radiate outwards from the adventitious wall of the heart, attaching the latter to the body wall.

The pericardial diaphragm is interrupted in the usual way in the intervals between the heart valves, the fibres converging on one another as they pass outwards on each side to the body wall. In the thorax where the dorsal vessel

leaves the hypodermis, the diaphragm becomes involved in forming an enveloping membrane which binds the oesophagus and aorta together.

The enclosure of the nerve cord in a sinus formed by a membranous curtain passing from side to side of the body must be mentioned in connection with the circulatory system. This curtain (Pl. II, fig. 11) for the greater part of its course, consists of an extremely fine membrane with flat nuclei. It extends from the prothorax to the seventh abdominal segment, the main body cavity being continuous with the neural sinus at these two points. In the prothorax the neural diaphragm is more strongly developed than elsewhere and is muscular. There seems to be no doubt that this acts as an accessory pulsating organ drawing the blood backwards. The accumulation of blood corpuscles at its posterior connection with the body cavity in fixed specimens is evidence in favour of this. Such an accessory blood conduit has been described in the Odonata by Tillyard (1917) and by Brocher (1919), who finds a ventral diaphragm in *Protoparce convolvuli* under which blood flows backwards in the abdomen.

Blood corpuscles are almost regularly spherical in form with spherical nuclei (Pl. III, figs. 15 and 16). They are best examined in fixed specimens in the regions of the heart valves where small groups of them are always found, and at the posterior end of the neural sinus where large numbers appear to congregate.

The *corpora allata* (Pl. II, fig. 8) are recognised as two round bodies, 35μ in diameter, closely attached dorso-laterally to the outer wall of the aorta, immediately behind the supra-oesophageal ganglion. Each corpus allatum consists of a nearly spherical mass of cells enclosed in a fine extension of the above-mentioned connective tissue sheath. The cells are almost spherical and arranged radially round a cytoplasmic core. In position, the corpora allata correspond to those of *Myrmica rufa*, as found by Janet (1899) and *Vespa*, *Bombus* and *Lasius* examined by Nabert (1913).

(5) *Fat-body.*

The fat-body consists of polygonal cells connected together in longitudinal sheets. They are highly vacuolated in the manner commonly found in this tissue in insects. The tissue has a regular distribution in the body (Pl. II, fig. 11), being found dorso-laterally to the gut and in the lateral compartment of the segments enclosed by dorso-ventral muscles. The largest part of the organ is that which lies lateral to the dorsal vessel (Pl. II, fig. 11) and dorsal to the alimentary canal. This mass possesses inner and outer layers adjacent to the gut and body wall respectively. Inner and outer layers are connected with one another along the sides of the dorsal vessel, and along the median lateral line of the body.

In the space enclosed are found the dorsal segmental longitudinal muscles (Pl. II, fig. 11), the outer layer of fat-body being interrupted at the places where these are attached to the hypodermis. The lateral masses of fat-body

are found in segmental arrangement in the abdominal region in close association with the oenocytes. No histological differentiation was found to exist between these different parts of the fat-body. Such an arrangement of this organ is commonly found in insects and calls for no further comment.

(6) *Larval oenocytes.*

The oenocytes (Pl. III, figs. 13 and 14) are arranged in segmental paired groups in the first eight abdominal segments. They find themselves for the most part in contact with the hypodermis from which they arose, in the lateral portion of each segment enclosed by dorso-ventral muscles. Each group consists of about 20 cells. The oenocytes are large subspherical cells about 30μ in diameter. The cytoplasm is vacuolated, some of the vacuoles adhering to the nucleus as found by Kreuscher (1922), others protruding from the surface like bubbles ready to burst (Pl. III, fig. 13). The secretory canals described by Kreuscher as radiating from the nucleus cannot be seen. The penetration of tracheoles with which the oenocytes are always in association often produces the appearance of such canals.

(7) *Musculature.*

The musculature was not studied in detail. At all places it is weakly developed and, except in the muscles of the body wall which are more strongly developed than elsewhere, only shows faint transverse striation. Muscle insertion on the hypodermis is not so clearly defined as in free-living insects, but agrees with Keilin's findings (1917) in that the attachment is effected by specially modified hypodermal cells.

(8) *Imaginal discs of the hypodermis.*

The imaginal discs develop late in larval life. In consequence they never sink deeply beneath the hypodermis, the peripodal membrane being reduced to a minimum. At the beginning of the last instar they are seen as more or less circular thickenings of hypodermis (Pl. II, fig. 11) and towards the end of this stage they become sunken slightly, the membrane turning slightly inwards over the disc as it passes outwards to the hypodermis of the body wall. The external opening of the wing disc is therefore very wide and easily seen through the transparent covering chitin.

The above description will serve for all discs. The following are the main imaginal discs of the hypodermis. In the head a pair of circular discs are found over the developing optic lobes of the brain. These give rise to the hypodermal structures of the compound eyes. They lie in a dorso-lateral position, and in front of them, but more ventrally placed, are the antennal discs of similar form. Simple discs develop within the larval mandibles, maxillae, and labium, as thickenings of the hypodermis of these organs. In the thorax, three pairs of ventral discs are found giving rise to the adult legs. Wing discs are laterally placed in the meso- and meta-thoracic segments.

An annular thickening of hypodermis round the anus is the source of cells which replace the posterior part of the larval hind-gut during pupation.

The remaining discs on the abdomen are those of the external genitalia. In the female these are found on abdominal segments 7, 8 and 9, and consist of one median and three pairs of hypodermal thickenings which give rise to the prominent ovipositor of the female. The ectodermal parts of the female genital ducts arise ventrally as a pair of invaginations on segment 7 close to the middle line. At this stage a pair of hypodermal swellings are found anterior to these incipient ducts. On segment 8 a pair of imaginal discs are found on the ventral surface more widely separated than those of segment 7. Between them is an unpaired hypodermal disc. On segment 9 the third and last pair of female genital discs is found. These are again more widely separated from one another than those of the preceding segment.

In the male larva the external genital aperture is wide and found on the ventral side of segment 10. It quickly divides into two short ectodermal invaginations, the paired ectodermal ducts of the testes. In front of the genital orifice a pair of imaginal discs is found on segment 9. A ventral thickening of hypodermis on segment 10 between the genital orifice and the anus, and which as yet shows little sign of its future bifid form, completes the larval male apparatus. The relation which these external genital discs bear to the adult anatomy will be a subject for further study. It is sufficient to notice the paired origin of the genital ducts, and to notice how closely the disposition of the imaginal discs of the female larva agrees with the arrangement in embryos of Orthoptera, Wheeler (1893) and Henneguy (1904). I can however find no discs on segment 8 in the male larva as found by Wheeler (1893) in the male embryo of *Ziphidium*.

(9) *Nervous system.*

The central nervous system is of the usual form found in insects. Behind the suboesophageal ganglion there are three thoracic ganglia and eight abdominals, of which the last is the largest consisting of three fused ganglia belonging to abdominal segments 8, 9 and 10.

The visceral nervous system, as examined by sections, consists of four ganglia. These are a median frontal ganglion (Pl. II, fig. 8) lying in front of the brain and from which a nerve passes to a small buccal ganglion between the dorso-ventral muscles of the buccal mass. From the frontal ganglion a nerve passes back along the oesophagus to the oesophageal valve where is situated the post-stomatogastric ganglion. On its course this nerve passes the oesophageal ganglion just behind the brain. It should be noted that the frontal ganglion is connected with the ventral nervous system by way of the circum-oesophageal commissures and not with the supraoesophageal ganglia as stated by Viallanes (1893).

VI. SUMMARY.

1. The life-history of *Phaenoserphus viator* is described.

Four larval instars are found, endoparasitic in the larvae of *Pterostichus niger*. At thee nd of the last larval instar the parasites, which may number as many as 45 in a single host, emerge, and while still attached, pupate without spinning a cocoon.

Adults may appear in August or September.

The effect of the parasite in inhibiting metamorphosis of the host is discussed.

2. The first observed larva is atracheate and incompletely segmented at first and is of the polypod type bearing paired prolegs on the body segments.

Subsequent instars are apodate.

The tracheal system develops progressively in the several instars, but only becomes functional in the final stage.

3. The anatomy of the larva is briefly described with the exception of the musculature.

Tracheal development is described. Gas only appears in the tracheae after the development of the tracheole cells puts the tracheae into communication with the body wall and other organs.

In the circulatory system an important accessory organ is the neural sinus, formed by the enclosure of the ventral nerve cord beneath a connective tissue curtain.

The imaginal discs of the hypodermis are briefly described, these being clearly defined in the head, thorax, and posterior abdominal segments.

The nervous system consists of a brain, suboesophageal ganglion and 11 ventral ganglia, the most posterior being tripartite. This system is connected with the sympathetic, by nerves passing from the cerebral commissures to a frontal ganglion which lies above the oesophagus and behind the labrum.

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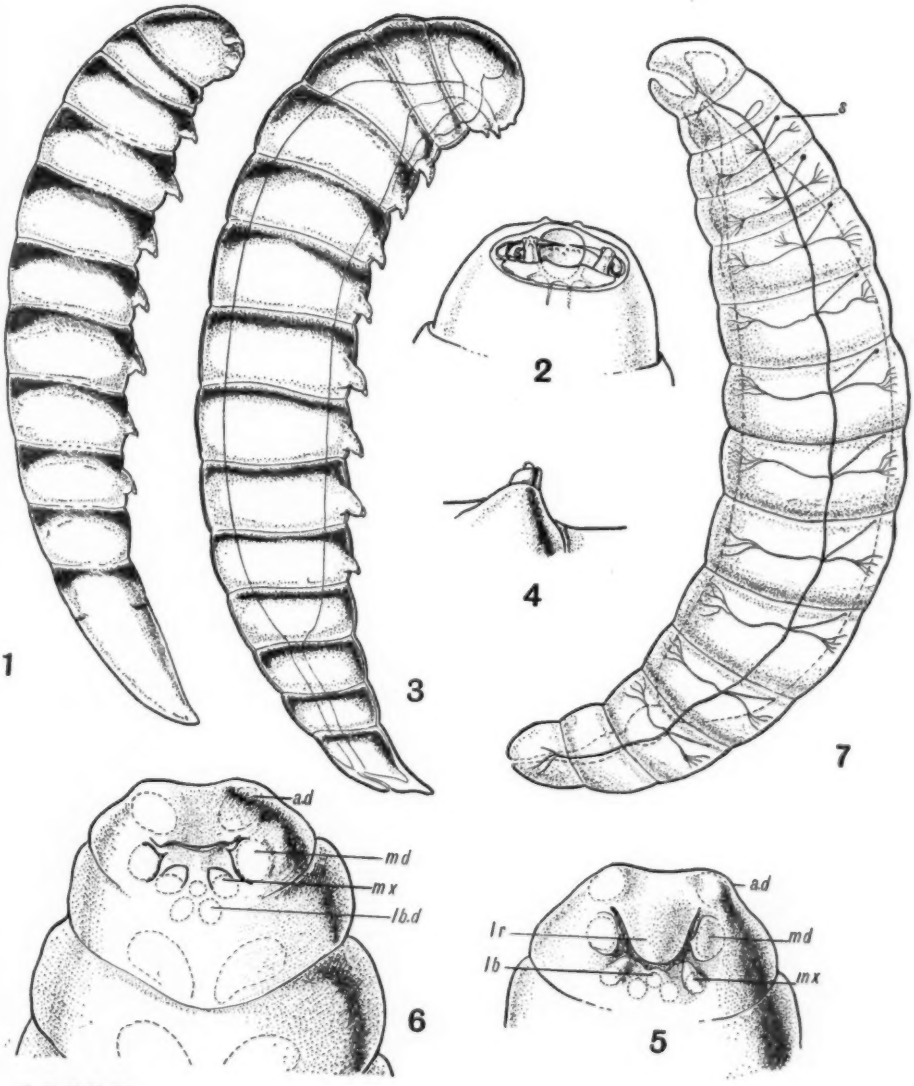

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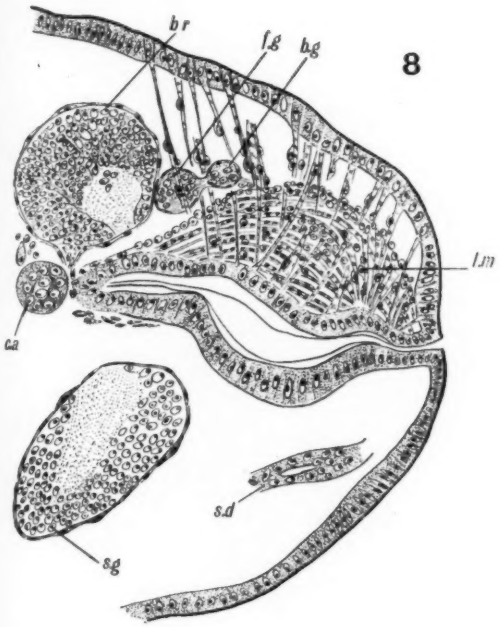

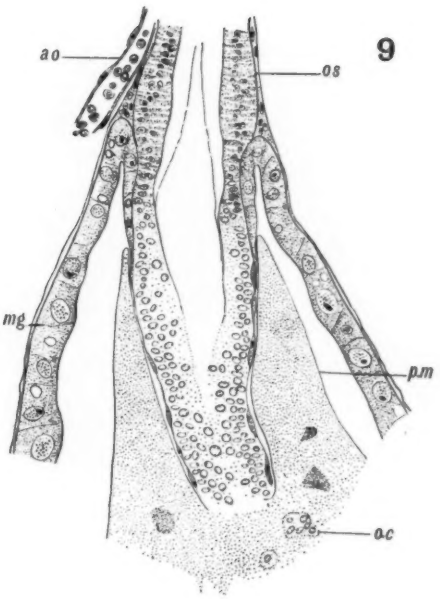

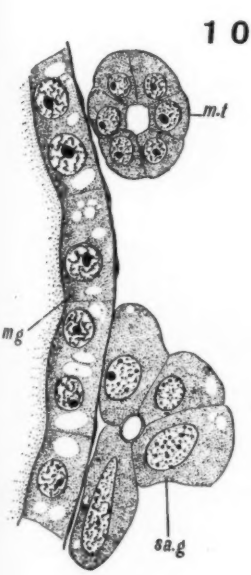

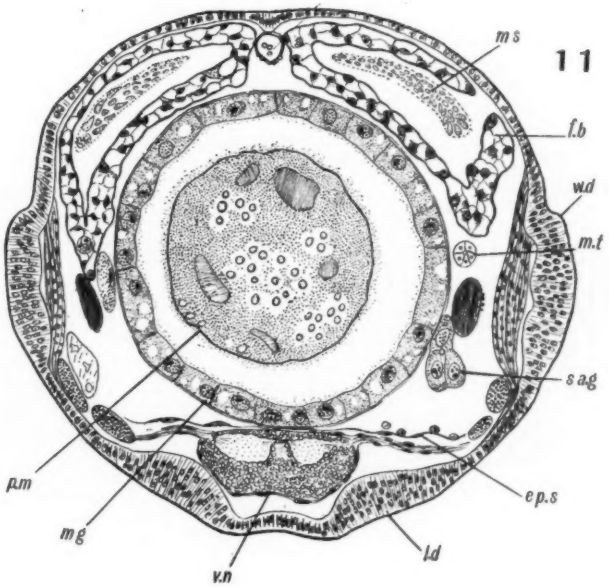

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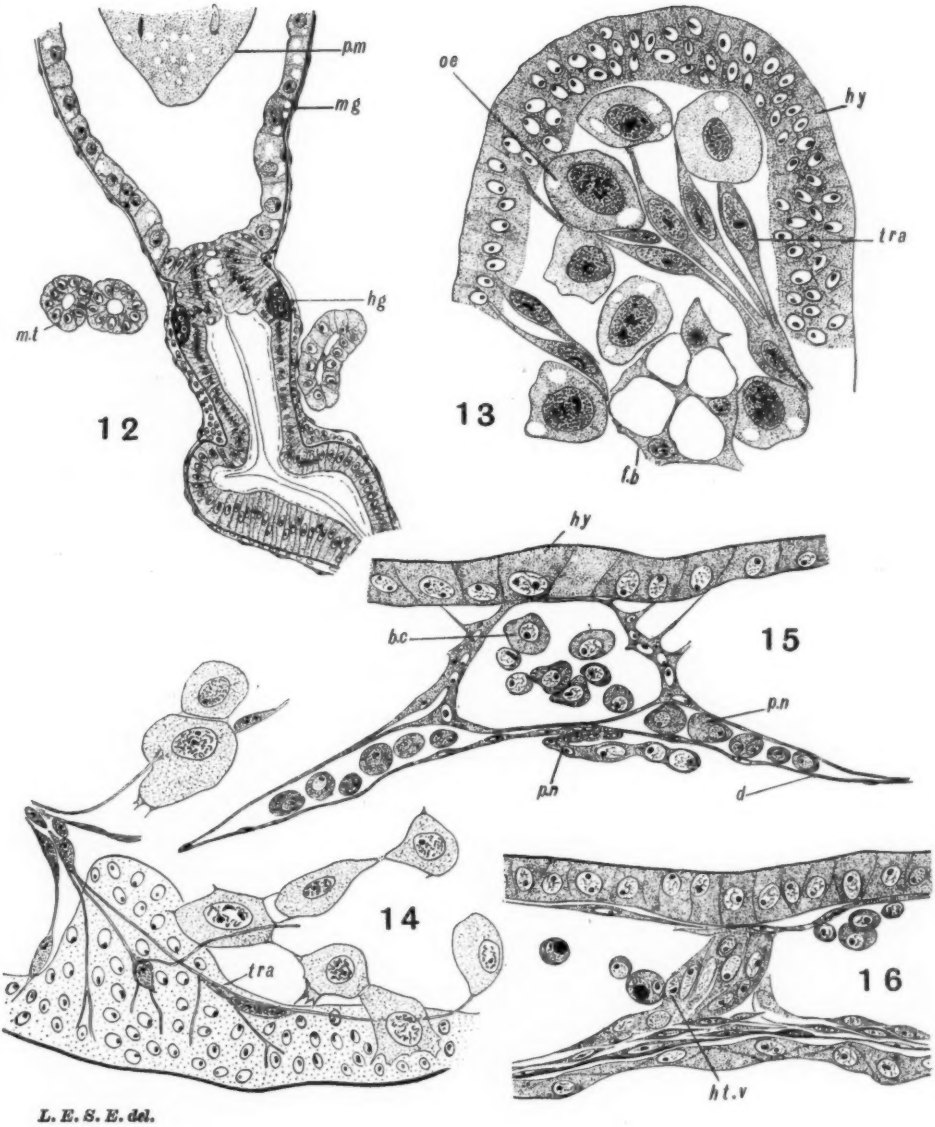

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EXPLANATION OF PLATES I—III.

PLATE I.

Phaenoserphus viator Hal.

Fig. 1. First observed larva showing the posterior macrosegment. Actual size 0·6 mm.

Fig. 2. Mouth-parts of first observed larva. Actual length of larva 0·6 mm.

Fig. 3. Completely segmented polypod larva. Actual length 0·7 mm.

Fig. 4. Proleg of polypod larva.

Fig. 5. Mouth-parts of late larva.

Fig. 6. Anterior region of prepupa illustrating mouth-parts and imaginal discs as seen by transparency.

Fig. 7. Last instar larva actual length 4·4 mm. showing tracheal system of one side.

PLATE II.

Phaenoserphus viator Hal.

Fig. 8. Longitudinal section through the head of mature larva.

Fig. 9. Longitudinal section through the oesophageal valve of the larva.

Fig. 10. Part of transverse section of mid-gut with salivary gland and malpighian tubule.

Fig. 11. Transverse section through mesothorax of larva.

PLATE III.

Phaenoserphus viator Hal.

Fig. 12. Longitudinal section through mid- and hind-gut of larva.

Fig. 13. Tangential section of body wall including oenocytes and tracheole cells.

Fig. 14. Tangential section of body wall of larva at the end of last instar showing tracheoles and oenocytes.

Fig. 15. Transverse section through heart showing pericardial diaphragm, pericardial nephrocytes and blood cells.

Fig. 16. Longitudinal section through heart and dorsal hypodermis, in region of a heart valve.

KEY TO LETTERING OF PLATES AND TEXT-FIGURE.

a.d., antennal disc.
ao., aorta.
b.c., blood cell.
b.g., buccal ganglion.
br., brain.
c.a., corpus allatum.
d., diaphragm.
ep.s., epineural membrane.
f.b., fat-body.
f.g., frontal ganglion.
hg., hind-gut.
ht.v., heart valve.
hy., hypodermis.
lb.d., labial disc.
l.d., leg disc.
l.m., labral muscles.
lr., labrum.
md., mandible.
mg., mid-gut.
m.t., malpighian tubule.
ms., muscle.
mx., maxilla.
o.c., oesophageal cells.
oc., oenocyte.
os., oesophagus.
p.m., peritrophic membrane.
p.n., pericardial nephrocytes.
s., spiracle.
sa.g., salivary gland.
s.d., salivary duct.
s.g., suboesophageal ganglion.
t., tentorial invagination.
tra., tracheole.
v.n., ventral nerve cord.
w.d., wing disc.

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THE TREMATODE PARASITES OF IRISH MARINE FISHES.

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(With a Map.)

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List of marine fishes examined at Galway, with observations on the Trematode parasites found; hosts arranged alphabetically	25
List of the Trematode parasites of marine fishes caught off the west coast of the Irish Free State; parasites arranged systematically	27
Summary	28

INTRODUCTION.

In the past, our knowledge of the Trematode fauna of marine fishes caught off the coast of Ireland has been extremely scanty and fragmentary, being confined to the works of Bellingham (1844) and Southern (1912).

Bellingham (1844), in his articles on Irish Entozoa, records the presence of a Trematode which he referred to *Distoma homoeostomum* Dies. in the stomach of the red gurnard (*Trigla pini* or *cuculus*), also a specimen of *Distoma microcotyle* Dies. from the intestine of the turbot (*Rhombus maximus*). The same author mentions the presence of *Distoma anonymum* Dies. in the alimentary canal of the haddock (*Gadus aeglefinus*) and the whiting (*Gadus merlangus*). *Distoma appendiculatum* (Rud.) was found by this author in the stomach of a sand eel (*Ammodytes lanceolatus*), while *Distoma reflexum* ? Crep. was found in the intestine of the lump-sucker (*Cyclopterus lumpus*). Bellingham also recorded a Trematode which he identified as *Distoma contortum* Rud. from the gills of the sun fish (*Orthagoriscus mola*), while in the intestine of this fish he found *Distoma nigroflavum* Rud.

A report of the Clare Island Survey containing a section on the Platyhelmia appeared in the *Proceedings of the Royal Irish Academy*. Southern (1912), in his introduction to this section, states, "with the exception of three species of Trematodes, all the members of the Phylum collected in the Clare Island district belong to the Class Turbellaria."

The three Trematodes mentioned by him are (*a*) *Phyllonella soleae* van Beneden and Hesse, which was found clinging to the skin on the ventral

surface of the common sole (*Solea vulgaris*), (*b*) *Tristomum molae* Blanchard, found attached to the gills of a sun fish (*Orthagoriscus mola*) taken near Achill by the late A. G. More, and (*c*) *Stephanophiala laureata* (Zeder), discovered in the intestine of the common trout (*Salmo fario*). The common trout cannot be regarded as a true marine fish, therefore the number of strictly marine fish examined by Southern is reduced to two.

The Scottish waters have been pretty thoroughly investigated by Thomas Scott and also by W. Nicoll.

Scott (1901 and 1904) records a number of Trematodes from fishes caught in the Moray Firth. Nicoll (1907, 1909), at the Gatty Marine Laboratory, investigated the Trematode parasites of fishes caught in St Andrews Bay. In 1910 he extended the investigation at the Millport Marine Biological Laboratory, examining fish caught off the east coast of Scotland. He states "that many species of Trematodes common in certain regions along the western shores of Europe never occur in the fishes obtained from the Scottish part of the North Sea." In 1913 Nicoll reported upon the Trematodes of deep-sea fish landed at Aberdeen.

The English waters have likewise received careful attention. Miss Lebour (1907) compiled a list of the fish Trematodes of the Northumberland coast. In the year 1909 James Johnstone described a Trematode found in a fish caught in Morecambe Bay. The fishes captured in the English Channel were examined for Trematode parasites by Nicoll (1914) who worked at the Plymouth Marine Biological Station.

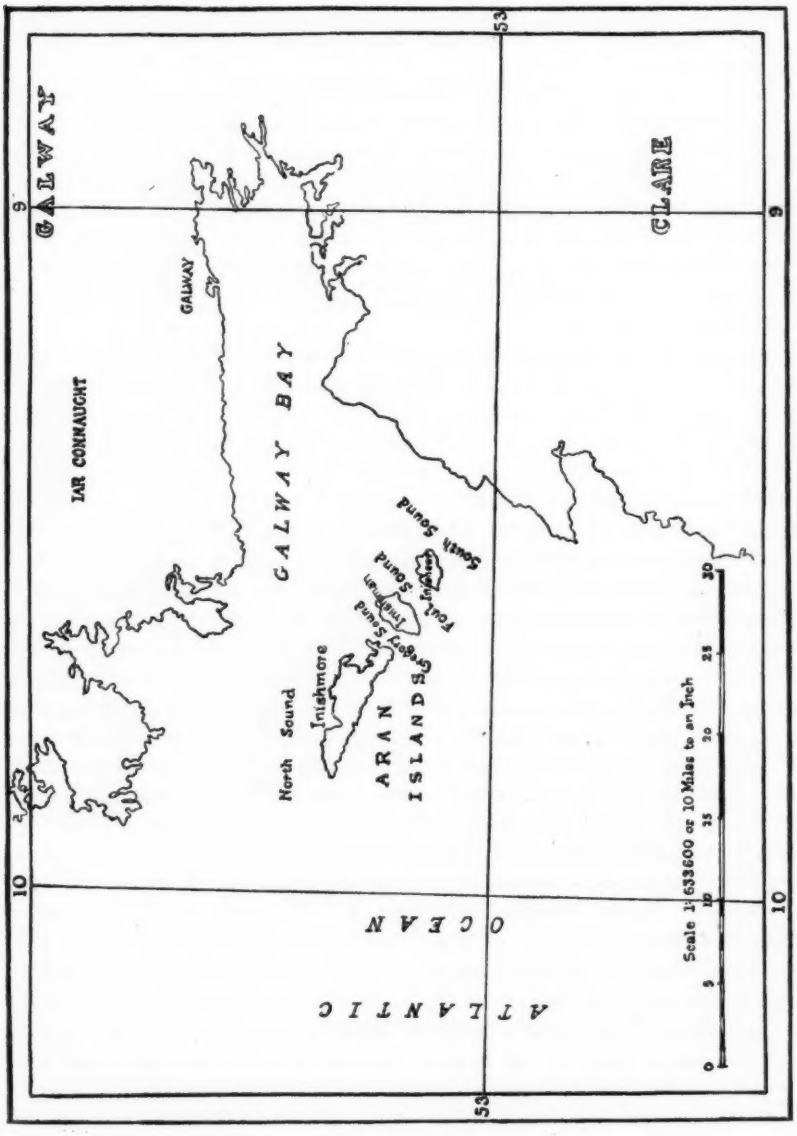
Information relating to the Trematode parasites of fishes caught off the Welsh coast has hitherto been of a somewhat fragmentary nature. The writer has therefore undertaken to investigate this problem, confining his attention to fishes caught in Cardigan Bay because of its geographical position, since it occupies that part of the Welsh seaboard which lies about midway between North and South Wales. Its marine fauna may be regarded therefore as typical for the Principality. The results of this work will be published shortly.

In both the New and the Old World investigators have devoted their attention to our subject. For a full appreciation of their work, the reader is referred to the literature appended to the paper by Nicoll (1915, pp. 372–8). Among the more important European papers may be cited those of P. J. van Beneden and Hesse, who chiefly examined fishes caught off the Belgian coast. Similar researches were conducted at Naples by Molin and in the Adriatic Sea by Stossich. Looss, Monticelli, Odhner, Rudolphi and Stossich record a number of Trematode parasites from fishes caught in the Mediterranean, and Olsson and Odhner examined respectively fishes caught off the Norwegian coast and in the Arctic Sea.

In the New World, Linton and Pratt record a number of Trematode parasites from marine fishes caught in different areas along the American seaboard, and a list of Trematodes from Canadian fishes has been compiled by Stafford.

This brief summary shows that the study of our subject so far as it affects

the British Isles, still remains incomplete, owing mainly to the absence of data from the Welsh and Irish coasts, more particularly the west coast of Ireland, where the broad Atlantic supplies one of the richest fishing grounds to be found around the British Isles.



Map of the area covered by the Survey.

My investigation was undertaken with a view to extending our knowledge of the subject, by examining the fishes caught off the coast of County Clare, County Galway, and off the Aran Islands. The following list should prove useful to future workers on the subject in the Irish Free State and elsewhere around the British Isles.

My work was conducted during the years 1926 and 1927 while Acting Professor of the Natural History Department at the Galway College of the National University of Ireland. The survey covers the area indicated by the Map which accompanies this paper.

For assistance in securing the necessary material grateful acknowledgments are due to Mr Martin McDonogh, T.D., of Galway, who offered me every facility to procure fish freshly captured by his trawlers, and also to the accomplished "skipper" of the trawler *Ocean Queen* for his unfailing courtesy and help.

In the following list the fish are named according to Dr J. Travis Jenkins' *The Fishes of the British Isles* (1925).

The habitat of the parasite is indicated for each host.

LIST OF MARINE FISHES EXAMINED AT GALWAY WITH OBSERVATIONS ON THE TREMATODE PARASITES FOUND.

(*Hosts arranged alphabetically.*)

N.B. New records are marked *.

1. *Arnoglossus megastoma* Day. Megrim.
   Two Trematodes were obtained; they are as yet undetermined.
   Miss Lebour (1907) examined two specimens of the Megrim captured off the Northumberland coast, but found no parasites.
2. *Conger niger* Cuv. (*C. vulgaris* Cuv.). Conger eel.
   - *Prosorhynchus aculeatus* Odhn. — Intestine
   - *Lecithochirium rufoviride* (Rud.) — Stomach
3. *Gadus luscus* Linn. Pout, bib or whiting pout.
   - *Hemiurus communis* Odhn. — Stomach
   - *Stephanochasmus caducus* Looss — Intestine and caeca
   - *Derogenes varicus* (Mueller) — Stomach
   - **Podocotyle atomon* (Rud.) — Intestine
   - *Dactylocotyle luscae* (van Ben. and Hesse) — Gills
4. *Gadus merlangus* Linn. Whiting.
   - *Octobothrium merlangi* (Kuhn) — Gills
   - *Hemiurus communis* Odhn. — Stomach
   - *Stephanochasmus pristis* (Deslongchamps) — Duodenum and caeca
5. *Gadus morrhua* Linn. (*G. callarias* Linn.). Cod.
   - *Dactylocotyle morrhuae* (van Ben. and Hesse) — Gills
   - *Hemiurus communis* Odhn. — Stomach
   - *Lepidapedon rachion* (Cobbold) — Intestine
   - **Prosorhynchus crucibulum* (Rud.) — Encysted in tissues of gills
   - *Derogenes varicus* (Mueller) — Stomach

6. *Gadus pollachius* Linn. Pollack.
   - *Dactylocotyle pollachii* (van Ben. and Hesse) — Gills
   - *Derogenes varicus* (Mueller) — Stomach
   - *Hemiurus communis* Odhn. — Stomach
   - *Podocotyle atomon* (Rud.) — Intestine
7. *Gadus virens* Linn. Coal-fish or saithe.
   - *Octobothrium denticulatum* Olss. — Gills
8. *Merluccius vulgaris* Flem. (*M. merluccius* Linn.). Hake.
   - *Derogenes varicus* (Mueller) — Stomach
   - *Hemiurus ocreatus* (Mol. of Olss.) — Stomach
9. *Molva molva* Linn. (*Molva vulgaris* Flem.). Common ling.
   - *Pterocotyle palmata* (Leuck.) — Gills
10. *Pleuronectes flesus* Linn. Flounder or white fluke.
    - **Lebouria varia* Nicoll — Intestine
    - *Hemiurus communis* Odhn. — Stomach
    - **Lecithaster gibbosus* (Rud.) — Intestine
11. *Pleuronectes limanda* Linn. Dab.
    - *Steringophorus furciger* (Olss.) — Intestine
    - *Zoogonoides viviparus* (Olss.) — Rectum
    - *Derogenes varicus* (Mueller) — Mouth
12. *Pleuronectes microcephalus* Donov. Lemon sole or lemon dab.
    - *Steringotrema cluthense* (Nicoll) — Intestine
13. *Pleuronectes platessa* Linn. Plaice.
    - *Zoogonoides viviparus* (Olss.) — Rectum
    - **Lecithaster gibbosus* (Rud.) — Intestine
    - *Derogenes varicus* (Mueller) — Stomach
    - **Hemiurus communis* Odhn. — Stomach
14. *Raja macrorhynchus* Raf. Flapper skate.
    - *Otodistomum cestoides* (van Ben.) — Stomach
15. *Raja maculata* Montagu. Spotted ray.
    - *Calicotyle kroyeri* Dies. — Cloaca
16. *Raja microcellata* Montagu. Painted ray.
    - No Trematodes discovered.
17. *Rhina squatina* Linn. Monk or angel fish.
    - *Pseudocotyle squatinae* van Ben. and Hesse — Skin
18. *Rhombus laevis* Rondel. Brill.
    - *Synaptobothrium caudiporum* (Rud.) — Stomach
    - *Derogenes varicus* (Mueller) — Mouth
19. *Scyllium canicula* Linn. Small spotted dogfish.
    - *Onchocotyle appendiculata* (Kuhn) — Gills
    - *Paracotyle caniculae* Johnstone — Gills and mouth
20. *Solea vulgaris* Quensel. Common sole.
    - *Podocotyle atomon* (Rud.) — Intestine
    - *Derogenes varicus* (Mueller) — Mouth and stomach
    - *Epibdella soleae* (van Ben. and Hesse) — Skin
21. *Sparus centrodontus* De la Roche. Common sea bream.
    - *Encotyllabe pagelli* van Ben. and Hesse — Gills
    - *Hemiurus communis* Odhn. — Stomach
    - *Derogenes varicus* (Mueller) — Stomach
    - *Steringotrema pagelli* (van Ben.) — Intestine

22. *Torpedo marmorata*. Common electric ray.
No Trematodes discovered.

23. *Trigla gurnardus* Linn. Grey gurnard.

Phyllocotyle gurnardi van Ben. and Hesse	Gills
Hemiurus ocreatus (Mol. of Olss.)	Stomach
Rhipidocotyle minima (Wagener)	Intestine
Prosorhynchus triglae Nicoll	Intestine
Stephanochasmus triglae Lebour.	Intestine
Derogenes varicus (Mueller)	Stomach

24. *Trigla hirundo* Day. *T. lucerna* Linn. Tub or yellow gurnard.

**Trochopus gaillimhe* Little	Gills
Phyllocotyle gurnardi van Ben. and Hesse	Gills
**Hemiurus communis* Odhn.	Stomach
**Helicometra pulchella* (Rud.)	Caeca
Derogenes varicus (Mueller)	Stomach

25. *Trigla lineata* Walb. Streaked gurnard.

**Helicometra pulchella* (Rud.)	Caeca

26. *Zeus faber* Linn. John Dory.

Synaptobothrium caudiporum (Rud.)	Stomach

LIST OF THE TREMATODE PARASITES OF MARINE FISHES CAUGHT OFF THE WEST COAST OF THE IRISH FREE STATE.

(*Parasites arranged systematically.*)

Order **DIGENEA** van Ben.

Sub-order PROSOSTOMATA Odhn.

Super-family DISTOMATA Zed.

Family **Allocreadiidae** Stoss.
- Sub-family Allocreadiinae Looss.
  - Genus *Podocotyle* (Duj., 1845)
    1. *P. atomon* (Rud.)
  - Genus *Lebouria* Nicoll
    2. *L. varia* Nicoll
  - Genus *Helicometra* Odhn.
    3. *H. pulchella* (Rud.)
- Sub-Family Stephanochasminae.
  - Genus *Stephanochasmus* Looss
    4. *S. triglae* Lebour
    5. *S. pristis* (Deslongchamps)
    6. *S. caducus* Looss
- Sub-family Lepocreadiinae Odhn.
  - Genus *Lepidapedon* Staff.
    7. *L. rachion* (Cobbold)

Family **Fellodistomidae.**
- Sub-family Fellodistominae Nicoll.
  - Genus *Steringophorus* Odhn.
    8. *S. furciger* (Olss.)
  - Genus *Steringotrema* Odhn.
    9. *S. pagelli* (van Ben.)
    10. *S. cluthense* (Nicoll)

Family **Zoogonidae.**
- Sub-family Zoogoninae Odhn.
  - Genus *Zoogonoides* Odhn.
    11. *Z. viviparus* (Olss.)

Family **Azygiidae.**
- Genus *Otodistomum* Staff.
  12. *O. cestoides* (van Ben.)

Family **Hemiuridae** Luehe.
- Sub-family Hemiurinae Looss.
  - Genus *Hemiurus* Rud.
    13. *H. ocreatus* (Mol. of Olss.)
    14. *H. communis* Odhn.
- Sub-family Sterrhurinae Looss.
  - Genus *Lecithochirium* Luehe
    15. *L. rufoviride* (Rud.)
  - Genus *Synaptobothrium* Linst.
    16. *S. caudiporum* (Rud.)
- Sub-family Lecithasterinae Odhn.
  - Genus *Lecithaster* Luehe
    17. *L. gibbosus* (Rud.)
- Sub-family Syncoeliinae Looss.
  - Genus *Derogenes* Luehe
    18. *D. varicus* (Mueller)

Sub-order GASTEROSTOMATA Odhn.

Family **Bucephalidae** Poche.
Sub-family Bucephalinae.
Genus *Rhipidocotyle* Dies.
19. *R. minima* (Wagener)

Sub-family Prosorhynchinae.
Genus *Prosorhynchus* Odhn.
20. *P. triglae* Nicoll
21. *P. crucibulum* (Rud.)
22. *P. aculeatus* Odhn.

Order **MONOGENEA** van Ben.

Family **Monocotylidae** Tasch.
Sub-family Calicotylinae Mont.
Genus *Calicotyle* Dies.
23. *C. kroyeri* Dies.
Sub-family Pseudocotylinae Mont.
Genus *Pseudocotyle* van Ben. and Hesse
24. *P. squatinae* van Ben. and Hesse
Sub-family Monocotylinae Gamb.
Genus *Paracotyle* Johnstone
25. *P. caniculae* Johnstone

Family **Tristomidae** Cobbold.
Sub-family Tristominae Braun.
Genus *Trochopus* Dies.
26. *T. gaillimhe* n.sp. Little
Sub-family Ancyrocotylinae Mont.
Genus *Epibdella* Blainv.
27. *E. soleae* (van Ben. and Hesse)
Sub-family Encotyllabinae Mont.
Genus *Encotyllabe* Dies.
28. *E. pagelli* van Ben. and Hesse

Family **Octocotylidae** van Ben. and Hesse.
Sub-family Octocotylinae Braun.
Genus *Octobothrium* F. S. Leuck.
29. *O. denticulatum* Olss.
30. *O. merlangi* (Kuhn)
Genus *Pterocotyle* van Ben. and Hesse
31. *P. palmata* (Leuck.)
Genus *Dactycotyle* van Ben. and Hesse
32. *D. morrhuae* (van Ben. and Hesse)
33. *D. pollachii* (van Ben. and Hesse)
34. *D. luscae* (van Ben. and Hesse)
Sub-family Plectanocotylinae Mont.
Genus *Phyllocotyle* van Ben. and Hesse
35. *P. gurnardi* van Ben. and Hesse
Sub-family Onchocotylinae Cerf.
Genus *Onchocotyle* Dies.
36. *O. appendiculata* (Kuhn)

SUMMARY.

A main feature of the data here presented is the number of new records. For the sake of brevity these are tabulated below, under their respective headings.

Another feature is the wide geographical distribution of a large percentage of the parasites herein recorded, particularly of such forms as *Zoogonoides viviparus* (Olsson), *Derogenes varicus* (Mueller) and *Hemiurus communis* Odhn. The former has been recorded for St Andrews, Millport, Aberdeen, Plymouth, Northumberland, Liverpool, Galway, and also for foreign seas. *Derogenes varicus* has been recorded for St Andrews, Millport, Plymouth, Aberdeen, Northumberland, Galway, and for foreign seas. *Hemiurus communis* has been recorded for St Andrews, Millport, Plymouth, Northumberland, Liverpool, Aberdeen, Galway, and for foreign seas.

This suggests that the intermediate host of the larval stages of these parasites must be some common marine form, in all probability an invertebrate, which itself enjoys a very wide geographical distribution.

A further feature is the tendency of certain parasites to infect a variety of fish, the more notorious being *Derogenes varicus* (Mueller) and *Hemiurus communis* Odhn. Of the twenty-six species of marine fishes examined at

Galway, eleven (42 per cent.) were found to harbour *Derogenes varicus*. The infected fishes were the bib (*G. luscus*), cod (*G. morrhua*), pollack (*G. pollachius*), dab (*Pleuronectes limanda*), plaice (*P. platessa*), tub (*Trigla hirundo*), grey gurnard (*T. gurnardus*), brill (*Rhombus laevis*), hake (*Merluccius vulgaris*), common sole (*Solea vulgaris*), common sea bream (*Sparus centrodontus*).

Hemiurus communis was found parasitising eight species of fish, *i.e.* 31 per cent. of the total number of species examined. The hosts affected were the bib (*G. luscus*), whiting (*G. merlangus*), cod (*G. morrhua*), pollack (*G. pollachius*), flounder (*P. flesus*), tub (*T. hirundo*), plaice (*P. platessa*), and the common sea bream (*S. centrodontus*).

At the moment it is a little premature to prophesy to what extent the discovery of encysted specimens of *Prosorhynchus crucibulum* (Rud.) in the gill tissues of the cod (*Gadus morrhua*) will assist in elucidating the life-history of this parasite. Mature specimens of *Prosorhynchus crucibulum* have previously been recorded as occurring in the alimentary canal of the conger eel (*Conger niger*). The writer discovered partly digested specimens of small cod to form a considerable bulk of the stomach contents of the conger eel.

SYNOPSIS OF THE NEW RECORDS MENTIONED IN THIS PAPER.

A. NEW RECORDS.

1. *New parasites.*

Parasite.	Host.
1. *Trochopus gaillimhe* Little	Recorded for the yellow gurnard (*Trigla hirundo*)

2. *New hosts.*

Host.	Parasite.
1. *Gadus luscus* Linn.	*Podocotyle atomon* (Rud.)
2. *G. morrhua* Linn.	*Prosorhynchus crucibulum* (Rud.) (encysted in gill-tissues)
3. *Pleuronectes flesus* Linn.	*Lebouria varia* Nicoll
4. *P. flesus* Linn.	*Lecithaster gibbosus* (Rud.)
5. *P. platessa* Linn.	*Lecithaster gibbosus* (Rud.)
6. *P. platessa* Linn.	*Hemiurus communis* Odhn.
7. *Trigla hirundo* Day*	*Hemiurus communis* Odhn.
8. *T. hirundo* Day	*Helicometra pulchella* (Rud.)
9. *T. lineata* Walb.	*Helicometra pulchella* (Rud.)

B. NEW RECORDS FOR THE BRITISH AREA.

These include the above total of ten records with the addition of the following:

Parasite.	Host.
1. *Dactylocotyle luscae* (van Ben. and Hesse)	*Gadus luscus* Linn.
2. *Octobothrium denticulatum* Olss.	*G. virens* Linn.
3. *Hemiurus communis* Odhn.	*Pleuronectes flesus* Linn.
4. *Synaptobothrium caudiporum* (Rud.)	*Rhombus laevis* Rondel
5. *Onchocotyle appendiculata* (Kuhn)	*Scyllium canicula* Linn.
6. *Podocotyle atomon* (Rud.)	*Solea vulgaris* Quensel
7. *Encotyllabe pagelli* van Ben. and Hesse	*Sparus centrodontus* De la Roche
8. *Lepidapedon rachion* (Cobbold)	*Gadus morrhua* Linn.

C. New records for Ireland.

With the exception of the following single record, all the records herein mentioned are new to Ireland. *Epibdella soleae* (van Beneden and Hesse) has previously been recorded by Southern (1912), who referred it to *Phyllonella soleae*.

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ON THE ENZYMES OF CERTAIN DERMATOPHYTES, OR RINGWORM FUNGI.

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(*From the Molteno Institute for Research in Parasitology, University of Cambridge.*)

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I. Introduction and historical.

A. *Introduction.*

The name Dermatophytes is a general term applied to fungi which cause superficial diseases of the skin, commonly called ringworms, in man and animals. According to Sabouraud's (1910) classification these fungi are distributed in the genera *Trichophyton*, *Microsporum*, and *Achorion*, which include fungi that attack both the hairy and glabrous skin. Later, the two genera *Epidermophyton* and *Endodermophyton* were formed for fungi which attack only the glabrous skin and never invade the hairs. Each of the three genera

was sub-divided by Sabouraud into a number of groups according to the manner in which the hairs are invaded, the size of the spores, cultural aspect, or whether they are parasites of man or animals or both. Recently new classifications of these fungi have been proposed, based on botanical or mycological characters. The most satisfactory of the new schemes appears to be that of Ota and Langeron (1923), who formed the new sub-family Closterosporeae of the Conidiosporaceae (Vuillemin, 1910) for the Dermatophytes, and divided them into the genera *Trichophyton*, *Sabouraudites*, *Epidermophyton*, *Grubyella*, *Bodinia*, and *Endodermophyton*. More recently Nannizzi (1926, 1927) has brought forward new evidence in favour of the view of Matruchot and Dassonville (1899, 1900, 1901) that the Dermatophytes are Gymnoascaceae.

As I have recently reviewed the whole subject (Tate, 1929), it is unnecessary to enter into further details regarding the classification here, or to deal with the morphology of the fungi. A peculiar feature of these fungi is their tendency to undergo, in culture, a change termed pleomorphism, which usually consists in the development, as an overgrowth of the mature colony, of a downy, sterile mycelium, which can be isolated in pure culture and never reverts to the normal or primary type of growth. This phenomenon is discussed at length in the review mentioned above, together with the significance assigned to it by various authors (Sabouraud, 1910; Grigoraki, 1925, 1927). Although the medical aspect of the subject and the morphology and cytology of the Dermatophytes have now been well investigated, the physiology of these fungi has been neglected completely by most workers.

The main object of the present work was to make a comparative study of the enzymes present in a number of Dermatophytes representing the chief groups in Sabouraud's system in order to find out whether or not his classification was supported by marked differences in the enzymic activities of the various groups. In a similar manner the normal and pleomorphic forms of one species were studied to see if the great morphological difference between the two forms was accompanied by a corresponding difference in their physiology.

B. *Historical.*

The earliest work on the physiology of ringworm fungi was carried out by Verujsky (1887), who made a comparative study of the morphology and physiology of *Trichophyton tonsurans* and *Achorion schoenleinii*. He found that a neutral or slightly acid medium is the most favourable for growth and that the optimum temperature is about 33° C. Spores of *Trichophyton tonsurans* are killed by heating in distilled water at 49° C. for ten minutes, but *Achorion* is more resistant and spores may germinate after heating for ten minutes at 50° C. Both cultures and spores are killed by minute traces of all the ordinary antiseptics. He also found that both liquefy gelatin, *Achorion* more rapidly; and that *Achorion* does not assimilate sugars in any medium but utilises large quantities of nitrogenous substances (urea); while *Trichophyton* utilises glucose with the formation of oxalic acid as an intermediate product, but does not

invert saccharose. On malt medium the ratio for *Trichophyton* of "weight of fungus : sugar consumed" is 1 : 2; but the presence of glycerine in the medium increases the ratio to 2 : 3.

Macfadyen (1894) showed that an enzyme which liquefies gelatin is present in *T. tonsurans*. It acts in both acid and alkaline media but is more active in the latter. It is present in gelatin liquefied by growth of the fungus and in both acid and alkaline broth infusions after growth. The optimum temperature for the reaction is about 37° C. The enzyme is very stable as it is still active in cultures three months old, but it is destroyed by heating at 100° C. for two minutes. He could not detect either diastase or invertase in this fungus; and found that it did not grow in milk and that milk was not curdled by cultures containing the enzyme. The enzyme did not act on fibrin. The fungus grew on keratin obtained from quills, and thereby rendered it more soluble in strong alkali, but a definite enzyme acting on keratin was not demonstrated.

Roberts (1894) found that hairs of man and animals were attacked to varying degrees when placed in growing cultures of *Trichophyton* and *Achorion*. Some species destroyed the cuticle and cortex simultaneously, while others first attacked the cortex and invaded the cuticle only later, or not at all. He proposed to utilise this "keratolytic" power for classifying Trichophytons. Later (1899), he found a proteolytic enzyme still active in cultures of *Trichophyton* six years old.

Bodin (1899) found that, for what he described as the oospora form of the *Microsporum* of the horse (*M. equinum* Bodin, in Sabouraud), the optimum temperature for growth is about 35° C., and that glucose, dextrin and maltose are assimilated in order of choice, but sucrose is not utilised. Later (1901) he and Lenormand found that the same fungus produces two enzymes in the culture fluid, one of which clots milk and the other dissolves the clot. The latter, casease, is most abundant in neutral peptone-glucose medium at the moment when the glucose has been consumed completely. The fluid containing casease also liquefies gelatin, and, to a much smaller degree, coagulates egg albumen and serum. In 1902 he found that *Achorion quinckeanum* also utilises glucose more readily than lactose or maltose; and that, after growth, the culture fluid contains casease, rennin (clotting milk), and gelatinase. Later (1907, cited in Sabouraud 1910) he described four proteolytic enzymes in *Achorion gypseum*: (1) trypsin, acting on coagulated albumen and more abundant in the pleomorphic form; (2) gelatinase, also more abundant in the pleomorphic form; (3) rennin, which coagulates milk; and (4), casease which dissolves the milk coagulum and is more abundant in the normal form.

II. Methods.

In selecting the species to be used in this work two considerations had to be borne in mind, (*a*) that they should be rapidly growing species, and (*b*) that as far as possible they should be representatives of the chief groups of Dermatophytes. The species finally selected were *Trichophyton tonsurans* (Malmsten,

1845) representing the endothrix, or human, Trichophytons of Sabouraud; *Sabouraudites radiolatus* (Sabouraud, 1910) representing the small-spored animal Trichophytons of Sabouraud; *S. audouini* (Gruby, 1843) representing the human Microsporums of Sabouraud; *S. lanosus* (Sabouraud, 1907) representing the rapidly growing animal Microsporums of Sabouraud; and *Grubyella schoenleinii* (Lebert, 1845) representing the Achorions of Sabouraud. Both the normal and pleomorphic forms of *S. radiolatus* were studied. In order to test the methods and to compare the behaviour of the Dermatophytes with that of ordinary saprophytic fungi: Bakers' yeast (*Saccharomyces cerevisiae*), *Aspergillus fumigatus*, and *A. niger* were used as control species in many of the experiments.

Method of growing the cultures. The cultures were grown in 250 c.c. Erlenmeyer flasks containing 50 c.c. of fluid medium, usually Sabouraud's glucose broth (glucose massée de Chanut 4 per cent., peptone granulée de Chassaing 1 per cent.), and were incubated at 30° C. until sufficiently advanced for the experiments. To reduce the risk of contamination during inoculation, short lengths of glass tubing about 6 mm. in diameter were inserted through the cotton wool plugs before sterilisation, the exterior end being closed with cotton wool rolled on a short length of stout wire and resembling a bacteriological "swab." The removal of the small plug permitted the flask to be inoculated through the narrow glass tube with very little risk of contamination.

Preparation of acetone powder. As far as possible it was desired to study the enzymes apart from the living mycelium, and the general method used was to prepare acetone powders from the cultures, resembling Buchner's "zymin" preparation from yeast (Onslow, 1923). By this method it was possible to reduce the mycelium to an exceedingly fine, dry powder, in which most of the enzymes were present uninjured, and which could be kept for weeks without deteriorating. It has also the great advantage from the comparative point of view, that cultures of different species, of the same age and grown under similar conditions, can be compared readily as regards the activity of any enzyme by testing similar weights of acetone powders prepared from the cultures. The method of preparation adopted was as follows: The mycelial mats from about six flask cultures were filtered off from the medium on a coarse glass filter. They were rinsed rapidly with distilled water to remove as much of the medium as possible, sucked dry on the filter, and roughly weighed in order to judge approximately the amount of acetone required in preparing the powder. It was found by experience that about 100 c.c. of acetone should be allowed for 10–15 grm. of mycelium. The mycelium was then ground up in a mortar with half the calculated amount of acetone. The mycelial mass was filtered off, and again ground up with half the remaining acetone. It was filtered off once more and ground up for the third time with the remaining acetone. Usually the mycelium was reduced to a fine powder by this time; and when it had been sucked as dry as possible on the filter, it was mixed up with about 25 c.c. of ether, which was then filtered off under slight pressure to com-

plete the drying. It was finally placed in a thermostat at 30° C. for several hours. If the preparation was successful, a fine, almost impalpable, powder was the result.

Alcohol powders. In certain cases for work on the respiratory enzymes, similar powders, but prepared with alcohol instead of acetone, were used. These powders will be called "alcohol" powders in distinction to acetone powders.

Triturated cultures. The alcohol and acetone powders served for most of the work, but in special cases other methods, such as grinding up cultures with sand in buffer solution or distilled water, with or without toluol, were used. These preparations will be described in connection with the experiments for which they were required.

Acetone powder suspensions. For use the acetone powders were generally made up in 1 per cent. suspensions in distilled water. As the fineness of the powders made them very difficult to wet, they were generally moistened with a little 50 per cent. alcohol before adding the water. The suspensions were divided into equal parts, one of which was either boiled or autoclaved to serve for controls. When it was necessary to keep the suspensions some time before use, a little toluol was shaken up with them to prevent the growth of micro-organisms.

Enzymes studied. Before proceeding to the description of the experimental work, a list of the enzymes which were dealt with will be given, arranged in the more or less arbitrary groups which are convenient for presenting the experimental results.

First is the group of respiratory, or oxidation and reduction enzymes, including oxidase, peroxidase, reductase, catalase and oxygenase. With this group it will be convenient to consider tyrosinase.

Next are the proteolytic enzymes, including pepsin, trypsin and keratinase. Here again it will be convenient to consider some enzymes which are not proteolytic. These are the lipolytic enzyme, lipase; and that hydrolysing urea or urease.

The last group is that of the carbohydrases, in which are included invertase, lactase, maltase, zymase, inulase and diastase. With them the glucoside-splitting enzyme amygdalase will be considered.

III. Respiratory, or oxidation and reduction enzymes.

Throughout the present work the three reagents guaiacum, benzidine, and "nadi" were used parallel with each other. The "nadi" reagent is prepared by mixing equal volumes of (1) $M/100$ solution of dimethyl-para-phenylene-diamine hydrochloride in 50 per cent. alcohol; (2) $M/100$ solution of alpha naphthol in 50 per cent. alcohol; and (3) a 0·1 per cent. solution of sodium carbonate. Solutions (1) and (2) should be freshly prepared and should not be mixed until immediately before use. The guaiacum reagent was prepared by making a saturated solution in absolute alcohol, and boiling the solution with

charcoal immediately before use to remove any organic peroxides that might be present. A saturated solution of benzidine in 50 per cent. alcohol was used.

It may be said at the outset that neither guaiacum nor benzidine gave a direct oxidase reaction with any of the fungi tested; but, with the addition of hydrogen peroxide, they were used for demonstrating peroxidase. For this purpose benzidine gave much more satisfactory results, those obtained with guaiacum being very uncertain. "Nadi" was the only reagent which gave an oxidase reaction. Catalase was revealed at the same time as peroxidase by the evolution of bubbles of oxygen on the addition of hydrogen peroxide, or by the addition of hydrogen peroxide alone.

A. *Peroxidase and catalase.*

Both peroxidase and catalase were found to be present in all the Dermatophytes tested and in *Aspergillus fumigatus* and *A. niger*, peroxidase being particularly strong in the two *Aspergilli*. These enzymes may be demonstrated in the living mycelium, but more constant results were obtained with the alcohol and acetone powder preparations. The peroxidase is thermostable. In order to save space the catalase and peroxidase reactions of the different fungi, and of the different acetone powder preparations, are tabulated together in Table I.

Table I.

Fungus	Powder	Peroxidase: Guai. + H_2O_2	Peroxidase: Ben. + H_2O_2	Catalase
A. fum.	Acetone	X	X	X
,,	Alcohol	X	X	—
A. niger	Acetone	—	X	X
S. rad. N.	Acetone I	0	X	X
,,	Acetone II	0	X	X
,,	Alcohol	X	X	X
S. rad. P.	Acetone I	X	X	X
,,	Acetone II	0	X	X
,,	Alcohol	X	X	X
S. lan.	Acetone I	X	X	—
,,	Acetone II	0	X	X
T. ton.	Acetone	0	X	X
G. schoen.	Acetone	0	X	X

The abbreviations for the specific names of the various fungi will be used throughout to save space in tabulated matter and are sufficiently obvious to require no explanation, except that *S. rad. N.* and *S. rad. P.* mean *Sabouraudites radiolatus* normal form, and *Sabouraudites radiolatus* pleomorphic form, respectively. Guai. = guaiacum reagent, Ben. = benzidine reagent, X = positive reaction, 0 = negative reaction.

B. *Indophenol oxidase.*

A direct oxidase reaction was not obtained with either guaiacum or benzidine but with the "nadi" reagent the presence of an indophenol oxidase was shown in all the fungi tested. This reagent is oxidised to indophenol blue in the presence of oxidase. The indophenol blue may be reduced to the leuco-base,

indophenol white, by reductase. The leuco-base may be oxidised by atmospheric, or molecular oxygen, independently of oxidase, to indophenol blue. Consequently, as was shown by Keilin (1927) for yeast, a positive oxidase reaction with the "nadi" reagent depends not only on the presence of oxidase, but also on the reductase activity. A positive oxidase reaction will not be obtained with the "nadi" reagent unless the activity of the oxidase is greater than that of the reductase, as otherwise no oxidase is available for oxidising the reagent. On the other hand, a negative reaction with this reagent is not significant unless the conditions are so arranged that the oxidase cannot be masked by the reductase. This may be accomplished by including controls in which the reductase is inhibited by heating to 52° C. for one hour, cooling to − 2° C., or the addition of a little ethyl urethane, all of which greatly inhibit the reductase but leave the oxidase almost completely uninjured. A trace of 1/1000 solution of KCN almost completely inhibits the activity of the oxidase.

In testing for indophenol oxidase, therefore, it is best to include the following series of controls: boiled, with the addition of KCN, with urethane, and heated to 52° C. for one hour. Experiments carried out on this plan failed to reveal the presence of oxidase in the acetone or alcohol powders prepared from any of the fungi, directly or after washing with distilled water. Positive reactions were obtained with cultures of *A. fumigatus* and *S. radiolatus* normal and pleomorphic forms, triturated with sand in phosphate buffer *p*H 7·3. A positive reaction was given only by the tube containing ordinary suspension, and the control tubes—boiled, heated to 52° C., with KCN, and with urethane —were all negative. (The technique of the experiments was the same as that described by Keilin (1927) for yeast.) This shows that the reductase activity, if any, is slight in these fungi as the oxidase reaction was not improved in the tubes in which the reductase was inhibited. The blue colour in the positive tubes did not fade on standing, which emphasises the slight reducing power of these fungi.

The indophenol oxidase is easily revealed in these fungi by placing portions of young mycelium in depressions in a white porcelain plate, covering with "nadi" reagent, and observing the change as compared with controls of boiled mycelium. In all cases the unboiled mycelium rapidly becomes blue owing to oxidase activity. Microscopical examination of the blue mycelium shows that there is no special localisation of the oxidase, and the hyphae are more or less uniformly stained blue. Sometimes granules are present which stain an intense blue, but they are equally common in boiled mycelium once autoxidation of the reagent has taken place, and are probably fat globules stained by the indophenol blue.

C. *Reductase.*

The presence of reductase is shown by the fact that if portions of mycelium which have given a positive reaction with "nadi" are placed on a slide and covered with a cover-slip, the blue colour fades completely after some time except around the margin of the mass, where, presumably, the diffusion of

oxygen from the surrounding fluid is sufficiently rapid to counterbalance the action of the reductase. The rapidity with which the blue colour is regained, once the cover-slip is removed, shows that the fading is due to the reduction of the indophenol blue to the leuco-base. The reduction to the leuco-base and reoxidation to indophenol blue may be repeated several times.

Attempts to show the presence of reductase in the acetone powders of all the Dermatophytes by incubating suspensions of the powders with methylene blue in Thunberg's vacuum tubes were completely negative. Before incubation the tubes were washed out with nitrogen and then evacuated to about 4 cm. pressure. It is evident, therefore, that the reductase activity of these fungi is very slight.

D. *Oxygenase and catechol system.*

According to Onslow (1923) and Onslow and Robinson (1926) the oxidase present in higher plants consists of an enzyme, oxygenase, which catalyses the autoxidation of an aromatic substance containing the ortho-dihydroxy group characteristic of catechol, and produces an organic peroxide, which is then decomposed by the enzyme peroxidase, with the formation of active oxygen. It is characteristic of oxidase plants that when the tissues are killed by direct injury or by such means as chloroform vapour, the oxygenase catalyses the autoxidation of aromatic substances present in the tissues with the formation of dark oxidation products, and the tissues of these plants rapidly turn brown or even black after death (Onslow, 1923). Recently Szent-Györgyi (1925) found that the oxygenase of potato produces an oxidation product of catechol which can blue guaiacum in the absence of peroxidase.

When dealing with cultures of these fungi killed by trituration with sand in buffer solution or distilled water containing toluol, in no case was any darkening of the mycelial pulp observed, even after standing for several days freely exposed to air; and suspensions of the pulp did not darken when incubated at 37° C. with the addition of a little catechol. This shows that the oxidase system in these fungi is quite different from the oxygenase-catechol system, and that the failure to give a direct oxidase reaction with guaiacum is not due to this system being present in an incomplete form owing to the absence of catechol.

E. *Tyrosinase.*

Only two of the fungi, *S. radiolatus*, normal and pleomorphic forms, and *A. niger*, were tested for tyrosinase. The experiments were conducted as follows: Cultures on Sabouraud's glucose broth were triturated with sand in phosphate buffer *p*H 7·3. The resulting suspension was distributed in four tubes: (*a*) 5 c.c. suspension only; (*b*) 5 c.c. fungus suspension + 5 c.c. tyrosine suspension; (*c*) 5 c.c. boiled fungus suspension + 5 c.c. tyrosine suspension; (*d*) 5 c.c. fungus suspension + para-cresol (Onslow, 1923). The tubes were incubated at 37° C., but all gave completely negative results.

IV. PROTEOLYTIC AND LIPOLYTIC ENZYMES AND UREASE.

A. *Pepsin and trypsin.*

The clotting of calcified milk provides a general test for proteolytic enzymes, and may be caused by pepsin, rennin or trypsin (Cole, 1920). Trypsin differs from the other two enzymes in gradually causing the disappearance of the clot. Pepsin and trypsin differ in the reaction of the medium at which they are active; pepsin acts in a very acid medium about *p*H 1·5; and trypsin in an alkaline medium, the optimum *p*H being about *p*H 8. They may also be distinguished by the products of the hydrolysis, which in the case of peptic hydrolysis are mainly peptones and polypeptides, while in tryptic hydrolysis the process is carried much further and results in a mixture of simple polypeptides and amino-acids.

In the following experiments the presence of a proteolytic enzyme is first demonstrated by means of the calcified milk test, and then further experiments are carried out to distinguish whether the enzyme present is pepsin or trypsin. For this purpose various methods were used, including testing for pepsin by the hydrolysis of carmine fibrin, and for trypsin by the hydrolysis of casein with the formation of free tryptophane; while albumen, gelatin and congo red fibrin were used in testing for pepsin and trypsin in acid and alkaline media respectively. The various methods will be described in connection with the appropriate experiments.

In all cases, unless the contrary is stated, 1 per cent. suspensions of the acetone powders in distilled water were used, and the controls were generally prepared with suspension which was either boiled or autoclaved to 120° C. Owing to the loss from evaporation during boiling, autoclaved controls were mostly used. The acetone powder suspensions are referred to as "enzyme suspensions."

Experiment 1. Detection of proteolytic enzyme by calcified milk.

The controls were prepared with enzyme suspension autoclaved to 120° C. The tubes contained 1 c.c. enzyme suspension and 2·5 c.c. calcified milk; and were incubated in a water bath at 38° C. A = tube with active enzyme, B = control tube with inactive enzyme, X = precipitate, XX = clotting.

Table II.

Fungus	6 min.	12 min.	24 min.
S. rad. N. A	XX	XX	XX
„ B	0	0	0
S. rad. P. A	X	X	XX
„ B	0	0	0
T. ton. A	0	0	X
„ B	0	0	0
S. lan. A	X	XX	XX
„ B	0	0	0
S. aud. A	0	X	XX
„ B	0	0	0
G. schoen. A	X	XX	XX
„ B	0	0	0

This experiment shows that all the species have a proteolytic enzyme, which is very active in all except *T. tonsurans*.

These acetone powders were then tested for pepsin with carmine fibrin (Cole, 1920) and Mett's egg-albumen tubes. The capillary tubes of egg white were prepared as described by Cole. The two experiments were similar except that the carmine fibrin was added to one series of tubes and the albumen to the other.

Experiment 2. Detection of pepsin by carmine fibrin and albumen.

The controls were autoclaved to 120° C. To each tube were added 1 c.c. enzyme suspension, 1 c.c. *N*/10 HCl, a little stained fibrin or two pieces of capillary albumen tubes about 1 cm. long, and a few drops of toluol. Incubated: water bath 37° C.

After 16 hours' incubation there was no hydrolysis of either the fibrin or albumen, showing that none of these species contains pepsin.

The absence of pepsin will be shown very clearly in the next experiment in which the same substrate is used in testing for both pepsin and trypsin in acid and alkaline media respectively. Fibrin stained with congo red was used as the substrate, and was prepared according to the directions given by Plimmer (1920).

Experiment 3. Detection of pepsin and trypsin by congo red fibrin.

The controls were autoclaved to 120° C. To the tubes of the trypsin series 1 c.c. enzyme suspension, 1 c.c. phosphate buffer *p*H 8, a little congo red fibrin and two drops of toluol were added. The tubes of the pepsin series differed only in having 1 c.c. *N*/10 HCl instead of the buffer *p*H 8. Incubated: water bath 37° C. XC = complete hydrolysis of the fibrin.

Table III.

	Trypsin *p*H 8				Pepsin *p*H 1·5	
Fungus	5 min.	30 min.	3 hr.	16 hr.	3 hr.	16 hr.
S. rad. N. A	X	XX	XXX	XC	0	0
„ B	0	0	0	0	0	0
S. rad. P. A	0	0	XX	XX	0	0
„ B	0	0	0	0	0	0
T. ton. A	0	0	X	X	0	0
„ B	0	0	0	0	0	0
S. lan. A	0	0	X	X	0	0
„ B	0	0	0	0	0	0
S. aud. A	0	0	X	X	0	0
„ B	0	0	0	0	0	0
G. schoen. A	0	0	X	X	0	0
„ B	0	0	0	0	0	0

The experiment very clearly shows the presence of trypsin and the absence of pepsin in all these species. It also shows that trypsin is present in varying amounts in the different species, *S. radiolatus* containing much more than the other species, and the normal form containing more than the pleomorphic of this species. The greater tryptic activity of the normal form of *S. radiolatus* is also shown by Experiment 1.

Similar results were obtained by testing for trypsin and pepsin with stained gelatin, in alkaline and acid media. This method has the disadvantage that the experiments cannot be conducted at a higher temperature than 30° C. Mr R. Hill, Biochemical Institute, Cambridge, suggested that stained gelatin suitable for these experiments was to be obtained ready for use in the form of the "tri-chrome-gelatin tissue" used in the three-colour process in photography. The magenta paper was used and gave excellent results, provided that the incubation temperature and time were well chosen. In acid solutions, when testing for pepsin, it cannot be used above room temperature; but in alkaline media when testing for trypsin, it may be used at 30° C. if the incubation period is not greater than 3–4 hours. Within these limitations it proved very convenient and gave very good results.

In the next experiment the same acetone powders were tested for trypsin by the hydrolysis of casein. In this method an alkaline solution of casein is incubated with the enzyme solution, and from time to time samples are withdrawn and tested for a precipitate by adding 1 per cent. acetic acid drop by drop. As long as unaltered casein, or paracasein, is present a dense precipitate is obtained; but if trypsin is present the casein is gradually hydrolysed to proteoses, peptones, and, finally, amino-acids, and no precipitate is obtained once the casein has been completely hydrolysed. Rennin and pepsin, in neutral or faintly acid solution, can hydrolyse casein to paracasein, but no further, so that this method differentiates between pepsin and trypsin (Cole, 1920).

Experiment 4. Detection of trypsin by the hydrolysis of casein solution.

In this experiment, in addition to the precipitation test with acetic acid, after 48 hours' incubation, the solutions were tested for free tryptophane by acidifying with acetic acid and adding a drop of bromine water (Onslow, 1923); and were also tested for peptones by means of the biuret reaction (Onslow, 1923). The casein solution was prepared in the manner described by Cole (1920). The usual enzyme suspensions were used and the controls were autoclaved to 120° C. The tubes with active enzyme suspension each received 2 c.c. enzyme suspension and 5 c.c. casein solution. The control tubes had half these quantities. A few drops of toluol were added to each tube, and they were incubated at 37° C. The precipitation test was applied to samples of about 0·5 c.c. withdrawn from time to time. The positive reactions with bromine gave a pink colour soluble in amyl alcohol, which is a characteristic test for free tryptophane. The biuret reaction was positive in both the controls and the active tubes, but was much stronger in the latter. P = precipitate, PP = dense precipitate, X = pink colour with bromine water (positive test for free tryptophane), or positive biuret reaction, Br. = bromine water test, Bt. = biuret test.

Table IV.

Fungus	1 per cent. acetic acid: 30 min.	1 hr.	2 hr.	15 hr.	40 hr.	Br. 40 hr.	Bt. 40 hr.
S. rad. N. A	P	0	0	0	0	XX	XXX
„ B	—	—	—	PP	PP	0	X
S. rad. P. A	P	P	P	0	0	XXX	XX
„ B	—	—	—	PP	PP	0	X
T. ton. A	PP	PP	P	0	0	X	XX
„ B	—	—	—	PP	PP	0	X
S. lan. A	PP	PP	P	P	0	X	XX
„ B	—	—	—	PP	PP	0	X
S. aud. A	PP	PP	P	P	0	X	XX
„ B	—	—	—	PP	PP	0	X
G. schoen. A	PP	P	P	0	0	XXX	XX
„ B	—	—	—	PP	PP	0	X

This experiment shows quite conclusively the gradual hydrolysis of the casein in the tubes with active enzyme suspension, and the production of free tryptophane in these tubes.

The results of Experiments 1–4 may be summarised by saying that an active proteolytic enzyme is present in acetone powder preparations of the fungi *S. radiolatus*, normal and pleomorphic forms; *S. lanosus*, *S. audouini*, *T. tonsurans* and *G. schoenleinii*. This enzyme acts in an alkaline medium and hydrolyses proteins with the production of free amino acids and consequently resembles trypsin. Pepsin is not present in any of these Dermatophytes. The proteolytic enzyme is most abundant in the normal form of *S. radiolatus*.

B. *Keratinase.*

The fact that the Dermatophytes can grow on hairs, either living or *in vitro*, and the remarkably luxuriant manner in which certain of them grow on feathers, as has been shown by Nannizzi (1926), suggest that they are able to hydrolyse keratin. Macfadyen (1894) has shown that some species can grow on pure keratin, but he did not demonstrate the presence of a keratolytic enzyme, and the experiments regarding the growth are not very conclusive.

Experiment 5. Detection of keratinase.

An experiment to try and detect the presence of a keratolytic enzyme in the acetone powders of the Dermatophytes was made. The method adopted consisted in incubating a suspension of pure keratin, which is insoluble in water, with the enzyme suspension, filtering the mixture, and applying the biuret test to the filtrate. If hydrolysis of the keratin had taken place, soluble proteins should be present in the filtrate and the biuret reaction should be positive, while if no hydrolysis had occurred the biuret reaction should be negative. Even after five days' incubation at 37° C. the filtrate gave a negative biuret test, showing that no hydrolysis of the keratin had taken place.

C. *Lipase.*

Three methods were used for the detection of a fat-splitting enzyme, or lipase. Milk, with the addition of a little phenol red as indicator, made alkaline

with sodium carbonate, affords a rough test for lipase, the presence of which is shown by the red colour changing to yellow owing to hydrolysis of the emulsified fat to fatty acids (Cole, 1920). This method is not very reliable, as, if trypsin is present, a slight hydrolysis of the casein takes place and the reaction becomes slightly more acid. More reliable results are obtained by using a mixture of cream and water instead of milk (Cole, 1920). Another method, which was found to be very good and which is probably more exact than the others, is that used by Avery and Cullen (1920) and Neill and Fleming (1927) in studying bacterial enzymes. In this method a 2 per cent. emulsion of tributyrin in phosphate buffer *p*H 7·8 is used as the substrate, and the lipolytic activity of the enzyme preparations is shown by the change in *p*H owing to the hydrolysis of the tributyrin into fatty acids. The amount of lipase present may be estimated by the change in *p*H in a given time. As this work on the Dermatophytes was purely comparative and qualitative, no very special precautions were taken in estimating the *p*H, all that was required was to determine the change in *p*H between the active and control tubes. For this purpose the B.D.H. "Capillator" method of *p*H determination was found to be very convenient and was quite accurate enough for work of this nature.

Experiment 6. Detection of lipase by phenol red cream.

A 10 per cent. emulsion of fresh cream in distilled water was used as substrate and was first heated to 100° C. in a water bath to destroy lactose fermenting bacilli. 1 c.c. enzyme suspension, 2·5 c.c. cream, 10 drops of phenol red and sufficient sodium carbonate to give a red colour, were mixed, divided into equal parts A and B, and B boiled to serve as a control. A little toluol was added to each tube and they were incubated at 38° C. in a water bath.

Table V.

Fungus	45 min.	2½ hr.	16 hr.
S. rad. N. A	X	X	XX
„ B	0	0	0
S. rad. P. A	X	X	XX
„ B	0	0	0
T. ton. A	X	X	XX
„ B	0	0	0
S. lan. A	X	X	XX
„ B	0	0	0
S. aud. A	X	X	XX
„ B	0	0	0
G. schoen. A	X	X	XX
„ B	0	0	0

A positive reaction of about equal strength was given by all the species. Experiments with phenol red milk gave similar results.

Experiment 7. Detection of lipase by tributyrin.

The controls were autoclaved to 120° C. Each tube contained 2 c.c. enzyme suspension, 2 c.c. of 2 per cent. tributyrin emulsion in buffer *p*H 7·8, and a few drops of toluol. Incubated: thermostat 37° C.

Table VI.

Fungus	pH Initial	pH 24 hr.	pH 48 hr.	pH change
S. rad. N. A	7·4–7·6	7·1	6·9	0·5
„ B	7·4–7·6	7·3–7·5	7·3–7·5	—
S. rad. P. A	7·4–7·6	6·9	6·7	0·8
„ B	7·4–7·6	7·5	7·5	—
T. ton. A	7·4–7·6	6·5–6·7	6·5	1·0
„ B	7·4–7·6	7·5	7·5	—
S. lan. A	7·4–7·6	7·1–7·3	7·1	0·4
„ B	7·4–7·6	7·5	7·5	—
S. aud. A	7·4–7·6	7·1	6·9	0·6
„ B	7·4–7·6	7·5	7·5	—
G. schoen. A	7·4–7·6	7·1–7·3	7·1	0·4
„ B	7·4–7·6	7·5	7·5	—

In all cases there is a marked hydrolysis of the tributyrin in the tubes with active enzyme. Other experiments in which additional controls consisting of (C) autoclaved enzyme suspension + buffer; and (D) active enzyme suspension + buffer gave similar results, except that the maximum reaction was not constantly given by the same species. Taking this fact into consideration with the phenol red milk and cream experiments, it appears that there is not very much difference in the lipolytic activity of the different species.

D. *Urease.*

Urease, which converts urea into ammonium carbonate, is known to be present in *A. niger* (Thom and Church, 1926). As Verujsky (1887) used urea as a source of nitrogen in a synthetic medium for ringworm fungi, the presence of this enzyme in the Dermatophytes was suspected and the following experiments were made to detect it. The method used is an application of the soya bean test for urea described by Cole (1920), by using urea in slightly acid solution as a test for the enzyme. A dilute solution of urea is added to the acetone powder suspension to be tested, together with a few drops of phenol red, and, if necessary, a little very dilute acetic acid to make the reaction slightly acid. On incubation, the presence of urease is shown by the yellow colour changing to red owing to the conversion of the urea into alkaline ammonium carbonate.

Experiment 8. Detection of urease.

The controls were autoclaved to 120° C. To each tube were added 1 c.c. enzyme suspension, 1 c.c. 1 per cent. urea solution, three drops of phenol red, and two drops of toluol. Incubated: water bath 37° C.

This experiment clearly shows that urease is present in the species *S. radiolatus*, in which there is much more in the pleomorphic than the normal form; *S. lanosus* and *S. audouini*, in both of which it is particularly abundant; and *G. schoenleinii*. *T. tonsurans* is completely devoid of urease in striking contrast to the other species.

The same results were given by a number of other experiments in which the amounts of enzyme and substrate were varied.

Table VII.

Fungus	40 min.	2 hr.	15 hr.
S. rad. N. A	0	X	X
„ B	0	0	0
S. rad. P. A	X	XX	XX
„ B	0	0	0
T. ton. A	0	0	0
„ B	0	0	0
S. lan. A	XX	XXX	XXXX
„ B	0	0	0
S. aud. A	XX	XXX	XXXX
„ B	0	0	0
G. schoen. A	X	XX	XX
„ B	0	0	0

V. CARBOHYDRASES.

The carbohydrate-splitting enzymes will be considered separately in the order invertase, maltase, diastase, inulase and zymase. Finally, it is convenient to deal with amygdalase in this section. The carbohydrases could not be studies in such detail as the proteolytic enzymes, owing to the fact that a comparatively large amount of enzyme suspension is required for these experiments; and also because most of the experiments require long incubation periods, often from 4–5 days.

In the course of the work on the carbohydrases it was found that after incubation the control tubes sometimes gave a slight reduction when boiled with Benedict's solution. This is due to the presence of a small amount of reducing substances in the enzyme preparations, which increases slightly during incubation, owing to autolysis, in tubes with active enzyme suspensions. To avoid this possible source of error when testing for invertase, lactase, maltase, diastase and inulase, additional controls were included in a number of the experiments, so that four tubes were used for each test as follows: A, active enzyme suspension + buffer; B, active enzyme suspension + substrate; C, inactive enzyme suspension + buffer; D, inactive enzyme suspension + substrate. In all cases where there was any reduction of Benedict's solution in the control tubes, it was so slight as to be quite negligible in comparison with that obtained where an active enzyme was present. Consequently, to economise space, these experiments with the three controls are not included in the tabulated material, as they did not influence the conclusions to be drawn from the experiments in which only one control, inactive enzyme + substrate, was employed.

A. *Invertase.*

Avery and Cullen (1920 *a*) found that the optimum reaction for the invertase of *Pneumococcus* is *p*H 7·0, and in the present experiments the saccharose was prepared in a phosphate buffer solution of this *p*H. Sometimes the saccharose was prepared as a 4 per cent. solution in distilled water, which was diluted with an equal volume of phosphate buffer *p*H 7·0 for use. The method used in testing for invertase consisted in incubating the enzyme suspension and buffered sugar solution at 37° C. and withdrawing samples at intervals and

testing for the presence of reducing sugars by boiling with Benedict's qualitative solution (Cole, 1920).

Experiment 9. Detection of invertase.

The controls were autoclaved to 120° C., and the saccharose prepared as a 2 per cent. solution in phosphate buffer *p*H 7·0. Each tube contained 1 c.c. enzyme suspension, 2·5 c.c. saccharose solution and a few drops of toluol. Incubated: thermostat at 37° C. They were filtered, and the filtrates boiled with Benedict's solution, after 24 hours' incubation. In no case was there any hydrolysis of the saccharose.

Other experiments also showed that none of these Dermatophytes is able to invert saccharose.

B. *Lactase.*

Experiments on the hydrolysis of lactose and maltose are difficult to carry out owing to the difficulty of distinguishing the products, glucose and galactose, from the unhydrolysed lactose or maltose remaining in the mixture. The method adopted was to make osazone tests (Onslow, 1923) after long incubation, and to try and distinguish glucosazone by its being less soluble than lactosazone and maltosazone, and by the microscopical appearance of the osazones obtained.

Experiment 10. Detection of lactase.

The controls were autoclaved to 120° C. and a 2 per cent. solution of lactose in phosphate buffer *p*H 7·0 was used as substrate. Each tube contained 3 c.c. enzyme suspension, 10 c.c. lactose solution, and five drops of toluol. They were incubated at 37° C. for four days, and then tested for osazones as described by Onslow (1923). They were examined at intervals for the appearance of a precipitate; and, when cool, the osazones were examined microscopically.

There was no marked difference in the rate of precipitation in the different tubes, and microscopically the osazones were indistinguishable in all the tubes. It therefore appears that none of these species has lactase.

C. *Maltase.*

The method used for the detection of maltase was similar to that used for lactase, but, in the final experiments, it was supplemented by first testing 1 c.c. of the filtrates for glucose by heating with Barfoed's reagent. Barfoed's reagent was first tried as regards suitability for distinguishing between glucose and maltose under such conditions, by testing pure sugar solutions of glucose and maltose, the maltose solution being of about the same concentration as the solution used in the experiments, and the glucose solution about four times more dilute. It was found that Barfoed's reagent readily differentiated between such solutions when equal volumes were heated for the same time with an equal quantity of the reagent; a definite reduction was obtained in the glucose tube and none in the maltose solution.

In the following experiments the appearance of precipitates of osazones during cooling is represented by P = slight precipitate; PP = precipitate; PPP = dense precipitate. The microscopical appearance of the osazones is indicated by M = pure maltosazone, m + g = mixed maltosazone and glucosazone, m + G = mixed maltosazone and glucosazone with the glucosazone predominating.

Experiment 11. Detection of maltase.

The controls were autoclaved to 120° C., and a 2 per cent. solution of maltose in phosphate buffer *p*H 7·0 (Neill and Fleming, 1927) was used as substrate. The tubes contained 3 c.c. enzyme suspension, 10 c.c. maltose solution and five drops of toluol. Incubated: 37° C. for four days. The osazone test was made in the same manner as in the experiment on lactase.

Table VIII.

Fungus	Precipitation			Microscopical
	10 min.	15 min.	1 hr.	
S. rad. N. A	0	0	PPP	m+g
„ B	0	0	PPP	M
S. rad. P. A	0	0	PPP	m+g
„ B	0	0	PPP	M
T. ton. A	PPP	PPP	PPP	m+G
„ B	0	0	PPP	M
S. lan. A	PP	PPP	PPP	m+G
„ B	0	0	PPP	M
S. aud. A	0	PP	PPP	m+G
„ B	0	P	PPP	M
G. schoen. A	0	PP	PPP	m+g
„ B	0	P	PPP	M

This experiment shows a definite difference in the rate of precipitation, and in the appearance of the osazones, in the active tubes of *T. tonsurans* and *S. lanosus*. In the other species there is an indication that some maltose has been hydrolysed, but the evidence is not conclusive.

Further experiments were made in a similar manner but the osazone test was checked by testing for glucose with Barfoed's reagent, prepared according to the formula given by Plimmer (1920).

Experiment 12. Detection of maltase.

The procedure was exactly the same as in the previous experiment, except that the incubation was limited to three days, and, before making osazone tests, 1 c.c. of each of the filtrates was heated to 100° C. for 3½ minutes with 5 c.c. of Barfoed's reagent. As this reagent cannot be used in buffered solutions the maltose solution was made up in distilled water.

The reduction with Barfoed's reagent agrees exactly with the indications of the precipitation, and the microscopical examination of the osazones. A strong reduction was obtained with the active tubes of *T. tonsurans* and *S. lanosus*, and a weak reduction in the active tubes of *S. radiolatus*, normal and pleomorphic forms, *S. audouini* and *G. schoenleinii*, while all the control tubes were negative. These experiments prove that maltase is present in all

Table IX.

Fungus	Barfoed's reagent	Precipitation 7 min.	15 min.	22 min.	Microscopical
S. rad. N. A	X	0	0	0	m+g
„ B	0	0	0	0	M
S. rad. P. A	X	0	0	0	m+g
„ B	0	0	0	0	M
T. ton. A	XX	0	PP	PPP	m+G
„ B	0	0	0	0	M
S. lan. A	XXX	PP	PPP	PPP	m+G
„ B	0	0	0	0	M
S. aud. A	X	0	PP	PPP	m+G
„ B	0	0	0	0	M
G. schoen. A	X	0	0	0	m+g
„ B	0	0	0	0	M

the fungi tested, but in very different amounts. *T. tonsurans* and *S. lanosus* are particularly rich in it; *S. audouini* and *G. schoenleinii* have less, but more than *S. radiolatus*, both normal and pleomorphic forms of which are very poor in maltase.

D. *Diastase.*

The method used for detecting diastase was to incubate starch in the form of either a 1 per cent. solution of soluble starch in distilled water or buffer, or a 1 per cent. starch paste in distilled water, with the enzyme suspension. From time to time drops were removed and tested for the disappearance of starch and formation of dextrins, by mixing with drops of iodine in potassium iodide on a white porcelain plate. In most of the experiments the iodine test was checked by boiling samples with Benedict's solution, either at intervals during the incubation or at the end of the experiment.

Experiment 13. Detection of diastase.

Avery and Cullen (1920 *a*) found that the optimum *p*H for *Pneumococcus* amylase is *p*H 7·0, and, consequently, in this experiment a 1 per cent. solution of soluble starch in phosphate buffer *p*H 7·0 was used as substrate. Controls were autoclaved to 120° C. 1 c.c. of the enzyme solution, 1 c.c. of starch solution and a few drops of toluol were added to each tube. They were incubated at 37° C. and tested with iodine and Benedict's solution at intervals. Immediately after mixing all the tubes were tested with iodine and all of them gave an intense blue colour. Colour reaction with iodine is indicated by B = blue, P = purple, RP = reddish purple, R = red, 0 = no colour change.

It is seen from this experiment that *T. tonsurans* and *S. lanosus* have a comparatively strong diastatic power, which is also well marked in *S. audouini* and *G. schoenleinii*, but is weak in both the normal and pleomorphic forms of *S. radiolatus*. Similar results were given by a large number of other experiments on diastase.

Table X.

Fungus	1 day		3 days		4 days	
	Iodine	Bened't.	Iodine	Bened't.	Iodine	Bened't.
S. rad. N. A	B	X	B	XX	RP	XXX
„ B	B	0	B	0	B	0
S. rad. P. A	B	X	B	XX	RP	XXX
„ B	B	0	B	0	B	0
T. ton. A	B	XXX	RP	XXXX	P	XXXX
„ B	B	0	B	0	B	0
S. lan. A	B	XXX	R	XXXX	R	XXXX
„ B	B	0	B	0	B	0
S. aud. A	B	XX	P	XXX	P	XXX
„ B	B	0	B	0	B	0
G. schoen. A	B	XX	P	XXX	P	XXX
„ B	B	0	B	0	B	0

E. *Inulase.*

In the experiments on inulase a 2 per cent. solution of inulin in phosphate buffer *p*H 7·3 (Avery and Cullen, 1920 *a*) was used for the substrate, and was tested for hydrolysis by boiling with Benedict's solution after incubation with the enzyme suspensions.

Experiment 14. Detection of inulase.

The controls were autoclaved to 120° C. Each tube contained 1 c.c. enzyme suspension, 2·5 c.c. 2 per cent. solution of inulin in buffer *p*H 7·3, and a few drops of toluol. They were incubated at 37° C. for four days.

Testing with Benedict's solution showed that no hydrolysis had occurred in any of the tubes, and therefore none of these species contains inulase. This conclusion was confirmed by other experiments.

F. *Zymase.*

Owing to the large amount of the acetone powders required for experiments on zymase, only one experiment on the Dermatophyte series of preparations could be carried out. The method used is that described by Onslow (1923) for detecting zymase by the production of carbon dioxide. Strong suspensions of the acetone powders were made in 10 per cent. glucose solution, divided into two equal parts A and B, of which B was boiled to serve as a control. The suspensions were then placed in short test-tubes fitted with rubber bungs through which passed a bent glass tube, one end of which communicated with the test-tube containing the suspension, while the other dipped under a solution of lime water in a second tube.

Experiment 15. Detection of zymase.

0·1 gm. of each of the acetone powders was suspended in 10 c.c. of 10 per cent. glucose solution and treated as described above. The tubes were incubated at 37° C. for 24 hours.

In no case was there any turbidity of the lime water, which shows that zymase is not present in any of the acetone powders.

G. *Amygdalase.*

Amygdalase hydrolyses the glucoside amygdalin with the formation of glucose, benzaldehyde and prussic acid, and is present in yeast and many species of *Aspergillus*, including *A. niger* (Thom and Church, 1926). The method used for detecting this enzyme consists in testing for prussic acid by suspending a strip of paper dipped in 1 per cent. picric acid and sodium carbonate in the vessel containing the hydrolysis mixture; and, after a suitable incubation period, testing the mixture for reducing sugars (glucose) by boiling with Benedict's solution (Onslow, 1923). In the presence of prussic acid the picric paper first turns orange yellow and finally red. In the following experiments X = reddening of the picric paper, or reduction with Benedict's solution.

Experiment 16. Detection of amygdalase.

Controls were autoclaved to 120° C., and a 2 per cent. solution of amygdalin in distilled water was used as substrate. To each tube were added 2 c.c. enzyme suspension, 1 c.c. amygdalin solution, a strip of picric paper, and a few drops of toluol. They were incubated in a water bath at 37° C. for 16 hours.

Table XI.

Fungus	Picric Paper 1 hr.	2 hr.	16 hr.	Benedict's 16 hr.
S. rad. N. A	0	X	XXX	XXX
„ B	0	0	0	0
S. rad. P. A	X	XX	XXX	XXX
„ B	0	0	0	0
T. ton. A	X	XX	XXX	XXX
„ B	0	0	0	0
S. lan. A	X	XX	XXX	XXX
„ B	0	0	0	0
S. aud. A	X	XX	XXX	XXX
„ B	0	0	0	0
G. schoen. A	X	XX	XXX	XXX
„ B	0	0	0	0

All the species have an active amygdalase but the normal form of *S. radiolatus* has considerably less than the pleomorphic form, or than any of the other species. Further experiments confirmed this and showed that there is no marked difference in the amount of amygdalase present in the other species.

VI. General discussion on the enzymes.

It has been shown that peroxidase and catalase are present in all the fungi studied—*A. fumigatus*; *A. niger*; *S. lanosus*; *S. radiolatus*, normal and pleomorphic forms; *S. audouini*; *T. tonsurans* and *G. schoenleinii*. In addition, all these fungi have an indophenol oxidase. The oxidase system in these fungi thus differs from the "catechol" system of higher plants (Onslow, 1923; Onslow and Robinson, 1926). Robinson (1924) has shown that there is a direct oxidase system in certain Basidiomycetes, which gives a direct oxidase

reaction with guaiacum. The system found in the fungi studied in the present work differs from this direct oxidase of the Basidiomycetes as it does not blue guaiacum in the absence of hydrogen peroxide. The system resembles the oxidase system which has been described recently by Keilin (1927) in yeast, and which consists of a peroxidase and an indophenol oxidase. In the case of yeast the oxidase can be revealed only when the reductase activity is inhibited; while in the fungi considered in the present work, the reducing power is very slight, and is not sufficient to mask the oxidase. Catalase, in varying amounts, is present in all the fungi mentioned above. Tyrosinase was not found in either of the species *S. radiolatus* or *A. niger*, which were the only ones tested.

The proteolytic enzyme, which is present in all the Dermatophytes investigated, very much resembles trypsin as it acts in an alkaline medium, gradually dissolves the clot which it produces in calcified milk, and hydrolyses intact proteins with the production of free amino-acids. A peculiar character of this enzyme, however, is that it does not hydrolyse coagulated egg albumin in either acid or alkaline medium. This enzyme corresponds to the four separate enzymes described by Bodin (1899, 1901, 1902, 1907) as casease, gelatinase, rennin and trypsin. Pepsin could not be detected in any of the Dermatophytes, although the proteolytic enzyme in *A. niger* and yeast closely resembles pepsin and not trypsin in its behaviour. The proteolytic enzyme is present to some extent in culture fluid after growth of the Dermatophytes, but it is impossible to say whether it is actually secreted into the medium or whether its presence is due to the autolysis or disintegration of older parts of the mycelium. The complex nature of the culture fluid makes it very unsatisfactory for use as an enzyme solution, so that the present work was almost entirely devoted to experiments with various preparations of the actual mycelium. The proteolytic enzyme may be precipitated from the culture fluid by adding twice the volume of acetone.

The proteolytic activity of the various Dermatophytes varies considerably; and *S. radiolatus* exhibits a marked predominance of proteolytic activity over all the other species.

The lipolytic activity of the Dermatophytes was not investigated previously. All of those studied were found to possess an active lipase which readily splits tributyrin into fatty acids. The amount of lipase in the different species is about equal.

Attempts to detect the presence of the keratolytic enzyme postulated by Macfadyen (1894) and Roberts (1894) failed completely in all the Dermatophytes.

Urease has been shown to be present in all the Dermatophytes used in this work with the very remarkable exception of *T. tonsurans*. It is particularly abundant in *S. lanosus* and *S. audouini*, fairly abundant in *G. schoenleinii* and in the pleomorphic form of *S. radiolatus*, but the normal form of *S. radiolatus* has very little.

Invertase, inulase, lactase and zymase are absent from all the species of Dermatophytes tested. Maltase and diastase are present in all to varying degrees. They are both most abundant in *T. tonsurans* and *S. lanosus*, fairly abundant in *S. audouini* and *G. schoenleinii*, but there is very little in both the normal and pleomorphic forms of *S. radiolatus*. The presence of diastase was denied by all previous workers, Verujsky, Macfadyen, Bodin and Roberts. Amygdalase is present in all species to about an equal extent, except for the normal form of *S. radiolatus* which has much less than the pleomorphic form, or than any of the other species.

The general carbohydrase and proteolytic activities of the various species appear to vary inversely to each other, those species which are rich in proteolytic enzymes being poor in carbohydrases and conversely. This is very obvious in *S. radiolatus* which has much the greatest proteolytic power, but is very poor in carbohydrases. This is very interesting because *S. radiolatus* is one of the group of small-spored ectothrix Trichophytons of Sabouraud (1910) which grow equally well on his peptone and sugar media, and species of this group are very prone to turn pleomorphic when grown on media containing high percentages of carbohydrates. Thus the presence of carbohydrates in the medium greatly influences the growth of this fungus although, to judge from the enzymic activity, the utilisation of carbohydrates is very small compared with that of proteins. Both *T. tonsurans* and *S. lanosus* are particularly rich in carbohydrases, while *G. schoenleinii* and *S. audouini* are species which have moderate proteolytic and carbohydrate-splitting activity.

The normal and pleomorphic forms of *S. radiolatus* behave similarly as regards respiratory enzymes, maltase and diastase, but they show marked differences in the proteolytic enzyme, and in urease and amygdalase. The normal form is considerably more active in proteolytic properties than the pleomorphic. The pleomorphic form has comparatively strong urease and amygdalase, which are both very weak in the normal form.

On the whole, it is apparent that the differences between the enzymic activities of the various species are only differences of degree. The results so far obtained, with a limited number of species, do not give a clear indication in support of any particular scheme of classification.

VII. Summary.

1. The enzymic activity was studied in the following Dermatophyte fungi: *Sabouraudites radiolatus*; *S. lanosus*; *S. audouini*; *Trichophyton tonsurans*; *Grubyella schoenleinii*. Both the normal and pleomorphic forms of *S. radiolatus* were studied comparatively.

2. An active proteolytic enzyme is present in all the species. This enzyme is active in an alkaline medium and can hydrolyse intact proteins (casein) with the production of free amino-acids (tryptophane), and is very similar to trypsin in its behaviour.

3. Pepsin is not present in any of these fungi.

4. The amount of the proteolytic enzyme present varies in the different species, and it is particularly abundant in *S. radiolatus*.

5. A keratolytic enzyme was not found in any of these fungi.

6. A lipolytic enzyme, lipase, which readily splits tributyrin into fatty acids, is present in all the species. It is about equally strong in all of them.

7. Urease is present in all, with the exception of *T. tonsurans*.

8. None of them contains invertase, inulase, lactase or zymase.

9. Maltase and diastase are present in all. They are strongest in *S. lanosus* and *T. tonsurans* and are weakest in *S. radiolatus*.

10. The species with most proteolytic activity have least carbohydrases, and conversely.

11. Amygdalase is present in all the species.

12. The normal form of *S. radiolatus* has greater proteolytic activity than the pleomorphic form. The pleomorphic form has strong urease and amygdalase, both of which are very weak in the normal form. Otherwise the enzymic activity of the two forms is similar.

I wish to express my sincere thanks to Dr D. Keilin and Mr F. T. Brooke, University of Cambridge, for help and advice; Dr Sabouraud, Saint Louis Hospital, Paris, for the gift of type cultures; the Medical Research Council, who bore the expense of the investigation; and the Department of Scientific and Industrial Research for a maintenance grant during the course of the work.

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A DESCRIPTION OF THE TREMATODE *CATOPTROIDES LACUSTRI* N.SP., WITH A REVIEW OF THE KNOWN SPECIES OF THE GENUS[1].

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(With 3 Text-figures.)

The genus *Catoptroides* was created by Odhner in 1902 to include those Gorgoderidae that have a discoidal posterior part of the body sharply set off by a groove from a slender stalk-like anterior portion, and have the testes nearly symmetrically arranged. Lühe (1909) added to these characters also the facts that the vitellaria are very small and irregular in outline and that the testes are deeply lobed.

Four species have been described as belonging to this genus; *C. spatula* Odhner, type species, and *C. spatulaeforme* Odhner, from African fish and *C. macrocotyle* Lühe, and *C. angulatus* (v. Linstow), from European fishes. The last mentioned, however, exhibits characteristics more in agreement with those of the genus *Phyllodistomum* Braun, and should not be included in this genus, as will be pointed out in the course of the discussion. On the other hand, Pearse in 1924 described a new Trematode from American catfish as *Phyllodistomum staffordi*, but as I shall point out in this paper it unquestionably is a member of the genus *Catoptroides* Odhner.

During the course of a study of parasites of Minnesota fish I found in the urinary bladder of the catfish *Ameiurus lacustris* (Walbaum, 1792), a hitherto undescribed species of Trematode. It has the above-mentioned generic characters of *Catoptroides* Odhner, but the specific characters are so different from any of those already described as to necessitate the creation of a new species for which I propose the name *Catoptroides lacustri*.

The fish from which this material was secured were caught in the St Croix River near Stillwater, Minnesota, in February 1928. Two of the eight fish examined were infected, one having four flukes, one of which was immature, while the other contained only one mature specimen.

[1] The writer wishes to express his appreciation to Dr W. A. Riley for his valuable suggestions and criticisms offered during the course of study of Minnesota fish parasites and the preparation of this paper.

Catoptroides lacustri n.sp. (Fig. 1).

The adult worms measure from 3·33 to 4·76 mm. in length, 2·11 to 2·55 mm. in width in the discoidal region and 0·72 to 0·93 mm. in the neck region, and 0·19 mm. in thickness. The anterior two-fifths of the body is narrow and sharply set off by a groove from the much broader, circular posterior portion. The margin of the discoidal part is distinctly crinkled.

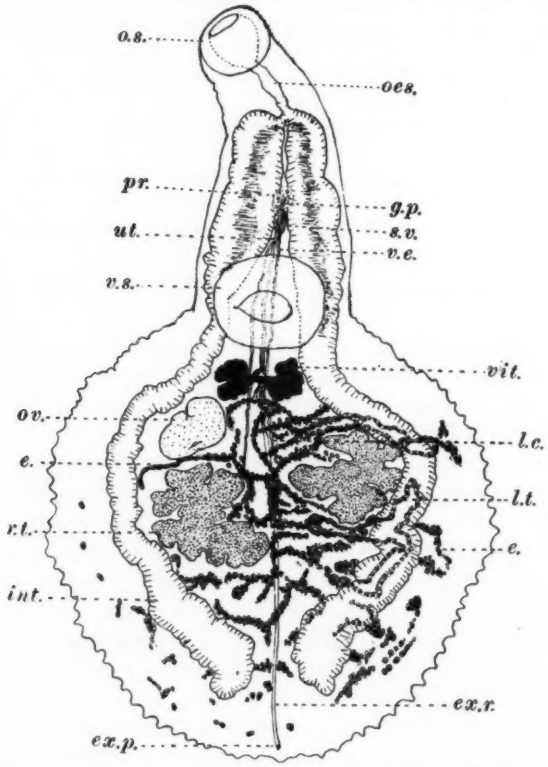

Fig. 1. *Catoptroides lacustri* n.sp., drawn with the aid of the camera lucida; ventral view. (× 33.) *e.* eggs; *ex.p.* excretory pore; *ex.r.* excretory reservoir; *g.p.* genital pore; *int.* intestine; *l.c.* Laurer's canal; *l.t.* left testis; *oes.* oesophagus; *o.s.* oral sucker; *ov.* ovary; *pr.* prostate; *r.t.* right testis; *s.v.* seminal vesicle; *ut.* uterus; *v.e.* vasa efferentia; *v.s.* ventral sucker; *vit.* vitelline gland.

Projecting from the surface of the cuticula are numerous inconspicuous papillae, which are more abundant near the suckers than elsewhere.

The oral sucker is terminal, measuring 0·30–0·40 by 0·28–0·39 mm. A pharynx is absent. The oesophagus is short, measuring about 0·25 mm. in length. The bifurcation of the oesophagus occurs anterior to the genital pore; from here the intestinal caeca extend back into the posterior fourth of the body. Their

course is curved convexly toward the lateral margins in the region of the gonads. The ventral sucker is located at the point of junction of the two body divisions (1·57 mm. from the anterior end), and measures 0·48–0·61 by 0·42–0·49 mm. The ratio of the oral to the ventral sucker is approximately as 3 : 5.

The excretory pore lies on the dorsum about 0·07 mm. from the hind margin and leads into a long slender bladder which extends unbranched up to near the vitellaria.

The testes are large, irregularly and deeply lobed bodies which measure 0·54–0·77 by 0·42–0·51 mm. They lie about in the middle of the discoidal part of the body occupying a latero-posterior position with respect to each other, the one on the same side with the ovary being the more posterior. Their long axes are not always in the same position, sometimes they run parallel to the main axis of the body while in other cases they are nearly transverse to it. The vasa efferentia pass out from the testes anteriorly, dorsal to the vitelline glands and acetabulum. Shortly anterior to the ventral sucker the two ducts come together and continue forward as the thin-walled vesicula seminalis, a bag 0·08–0·1 mm. in length and usually filled with spermatozoa. From here a rather thick-walled pars prostatica, 0·06 mm. in length, extends forward and downward into an enlarged genital sinus. A cirrus is absent. The genital sinus communicates with the outside through the genital pore which lies about two-thirds from the anterior end, slightly behind the junction of the intestinal rami. Surrounding the anterior part of the pars prostatica (vas deferens) lies the prostate.

The large ovoid ovary may lie on either the right or left side, immediately anterior to one of the testes. It is slightly notched on either side and measures 0·34–0·38 by 0·22–0·28 mm. The vitellaria consist of paired glands lying in a median position anterior to the gonads and directly behind the acetabulum. They are distinctly lobed and considerably smaller than the ovary; they lie quite close together, being *ca.* 0·085 mm. apart and also about as far posterior to the sucker. A shell gland and a seminal receptacle are absent. Laurer's canal opens on the dorsal surface to the left of the median line some distance behind the vitellaria. The uterus is extensively coiled, lying largely between the intestinal rami and testes and forward up to the vitellaria, but not infrequently passing also beyond the caeca. It eventually makes its way anteriorly passing beneath the seminal vesicle to open into the genital sinus. The eggs are in a more or less continuous chain and are ovoid in shape measuring 0·036 by 0·024 mm. in size.

HOST: *Ameiurus lacustris* (Walbaum, 1792), the great fork-tailed catfish of America; in urinary bladder.

TYPE: Cat. No. 8024, U.S. National Museum; paratypes in Department of Zoology, University of Minnesota, and in author's collection.

The taxonomic position of this new species is still further established in the following comparative review of the known species of the genus *Catoptroides* Odhner.

REVIEW OF THE KNOWN SPECIES.

Catoptroides spatula Odhner; type.

1902 *Phyllodistomum spatula* Odhner. *Centralbl. f. Bakt. Parasitenk.* Orig. **31**, 66.

1902 *Catoptroides spatula* Odhner in Looss, *Zool. Jahrb.*, Abt. Syst. Geog. u. Biol. **16**, 861.

Odhner's description of this species is as follows: Length about 5–5·75 mm. The body is divided into a conical anterior portion with a length of about one-third the total body length and a circular posterior portion with a diameter of about 3·3–3·6 mm. At or near the junction of the two body regions lies the acetabulum. This is slightly larger than the oral sucker (in one case 0·52 against 0·48 mm.). A pharynx is absent. The intestinal rami commence at the beginning of the second third of the neck. Testes lie symmetrical, because the posterior has moved forward with respect to the anterior; they are considerably lobed. The ovary is relatively large and orbicular; decidedly unnotched. The vitellaria are small, lobed. Coils of the uterus lie behind the gonads, but occasionally they cross the intestinal rami. The eggs are about 0·03 mm. long. The genital pore lies about two-thirds the length of the neck from the anterior end.

HOST: *Bagrus docmac* and *B. bayad*, catfish from the Nile; in urinary bladder.

C. lacustri agrees with *C. spatula*, the type of the genus, in that the slender neck is distinct from the circular posterior portion; the vitellaria are small and lobed; the testes are deeply lobed; the genital orifice is in both species median and about two-thirds the length of the neck from the anterior end. The absence of the pharynx is a sub-family character. It differs from the type in being smaller; in showing a greater difference in the relative size of its two suckers; in that the eggs are larger and more elliptical in shape; in that the margin of the discoidal part is crinkled; in that the testes are less symmetrical, but still considerably more so than is typical for the phyllodistomes; and in that the uterine coils lie more forward, reaching up to the vitelline glands. These differences are specific in nature and justify the establishment of a new species.

Catoptroides spatulaeforme Odhner.

1902 *Phyllodistomum spatulaeforme* Odhner. *Centralbl. f. Bakt. Parasitenk.* Orig. **31**, 67.

1902 *Catoptroides spatulaeforme* Odhner in Looss, *Zool. Jahrb.*, Abt. Syst. Geog. u. Biol. **16**, 861.

According to Odhner this species differs from *C. spatula* in that the testes are not symmetrical, but take a position half-way between it and those of the species belonging to the genus *Phyllodistomum* Braun, 1899. In the latter genus the testes take a definite and marked latero-posterior position. Besides the

above character *C. spatulaeforme* Odhner differs from the type species in that the worm is relatively smaller, being 4·75 mm. in length and 2·8 mm. in width. The ventral and oral suckers measure 0·4 mm. and 0·35 mm. respectively. The testes lie close to the ovary and the vitelline glands. The ovary is kidney-shaped. The vitellaria are irregularly rounded, but without definite notches. Uterine coils as well as the genital pore are like those in the type species. Eggs measure 0·028 mm.

Host: *Malapterurus electricus* Lacep., the electric catfish of the Nile; in urinary bladder.

This species agrees with *C. lacustri* in that the testes are latero-posterior in position; they are similar in size. *C. lacustri*, however, has a higher ratio between the two suckers, the margin of its body is crinkled, the eggs are larger and the uterine coils extend more forward.

Catoptroides macrocotyle Lühe.

1909 *Catoptroides macrocotyle* Lühe. *Die Süsswasser-fauna Deutschlands* (Brauer, Jena), Heft 17, *Parasitische Plattwürmer*, M. Lühe, I. Trematodes, p. 100.

Lühe describes this species as taken from the ureters, very seldom from the bladder, of a number of species of Cyprinidae. Usually about 5 or 6 occur in a single host, but as many as 40 have been taken from one fish. *C. macrocotyle* Lühe range from 2 to 5 mm. in length. The acetabulum lies near the middle of the body length and is about twice as large as the oral sucker. (In sexually immature forms with the body length less than 1 mm. and the acetabulum lying even posterior to the middle of the body, this sucker is as small or even smaller than the oral sucker. Otherwise in larger forms their ratios vary between 11 : 6 and 5 : 2.) In young forms the posterior part of the body is from 4 to 5 times as wide as the anterior portion; in sexually mature forms about 3·5 times as broad and barely one-third as wide as the total length, with a pointed posterior end. The tender cuticula varies from 0·0015 up to 0·003 mm. in thickness. The branching of the intestine occurs about two-thirds the way from the anterior end to the acetabulum. The ovary is 3–6 (usually 4) lobed, lying behind one of the vitellaria (right ?). The testes are considerably elongated (0·5 mm.) and deeply lobed, lying somewhat behind and to the side of each other. Eggs enlarge during their development in the uterus from 0·032 by 0·018 to 0·054 by 0·036 mm.

Hosts: *Carassius carassius* (L.), *Barbus barbus* (L.), *Gobio gobio* (L.), *Scardinius erythrophthalmus* (L.), *Leuciscus rutilus* (L.), *Leuc. cephalus* (L.), *Leuc. idus* (L.), *Aspius rapax* Agass., *Abramis brama* (L.), and *Abr. björkna* (L.), from central Europe; in urinary ducts, seldom in bladder.

In certain respects this species appears to be markedly different from the type species so that a more complete and comparative study, giving illustrations, is necessary to clear certain points in question. Lühe makes no mention

of the presence or absence of a groove separating the slender neck from the posterior region, a generic character. Although it conforms to the generic type in the lobed condition of the testes, the descriptive terms used to show their relative position may be interpreted to apply to those of either genus, *Catoptroides* or *Phyllodistomum*. *C. macrocotyle* Lühe is furthermore striking in that it possesses a relatively longer neck than any one of the other species (I take it that the neck reaches up to the acetabulum as it does in other species, although no definite statement exists to this effect); the ratio between the oral and ventral sucker is higher; the oesophagus is relatively longer; the ovary is much more lobed; the eggs are larger; the posterior end is more definitely pointed. These latter characters are only specific in nature and would not alter its generic relationship.

Catoptroides staffordi (Pearse).

1924 *Phyllodistomum staffordi* Pearse. *Trans. Wisc. Acad. Sci. Arts and Letters*, **21**, 154, Figs. 3–3 A.

A. S. Pearse, in 1924, described a new species of Trematode (Fig. 2) taken from the bladder of catfish of Wisconsin waters. He placed this species into the genus *Phyllodistomum* Braun. It is characterised by its body length being "4·1 mm.; width 2·98 mm. Diameter of oral sucker, 0·38 mm.; acetabulum, 0·52 mm. Egg, 0·027 by 0·021 mm. Anterior quarter of body narrow, flattened, sharply set off from the posterior portion by a groove. Behind the middle of the acetabulum the body is discoidal, being nearly circular in outline; the margins are thin, but not crinkled, and are covered with minute papillae. The oesophagus is short—a little less than the diameter of the oral sucker in length. The intestinal rami are quite wide (about 0·3 mm.) and extend to the posterior eighth of the body.

"The genital pore is nearer the anterior margin of the acetabulum than the junction of the intestinal rami, and is median. The testes are nearly equal in size, the left being a little larger, irregularly lobate, and lie on either side of the body, latero-posterior to the vitelline glands. The left testis is a little more posterior than the right, and the ovary lies between it and the nearest vitelline gland. The testes are small, measuring about 0·42 mm. in diameter. There is a seminal vesicle, about 0·5 by 0·7 mm.

"The ovary is elongate, lobate, and measures about 0·25 mm. in length. The two vitelline glands are each composed of about eight oval lobules. They are 0·115 mm. apart and about the same distance from the posterior margin of the acetabulum. The uterus is coiled between and posterior to the testes, largely between the intestinal rami. It does not come close to the lateral or posterior margins; its coils do not lie very close together and do not contain a continuous series of eggs, but discontinuous groups" (Pearse, 1924).

This species has the distinct generic features of *Catoptroides* Odhner. As he describes it, the "anterior quarter of the body narrow, flattened, sharply set off from the posterior portion by a groove"; "the testes are nearly equal

in size,. . .irregularly lobate" and "the left testis is a little more posterior than the right"; these are the very characters that place it in the genus *Catoptroides* Odhner, and it should, therefore, be named *C. staffordi* (Pearse, 1924).

HOST: *Ameiurus nebulosus* (Le Sueur) and *A. melas* (Raf.), American catfish; in urinary bladder.

In generic characters this species is in agreement with the type except in that the testes are not entirely symmetrical. It is distinct from any of the other species in that the eggs are smaller and occur in conspicuous groups in

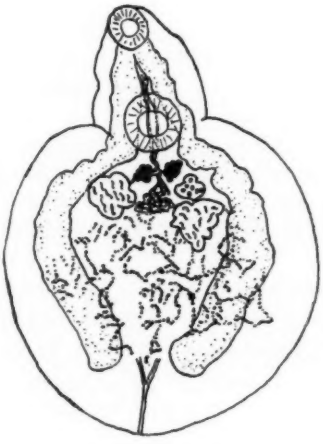

Fig. 2.

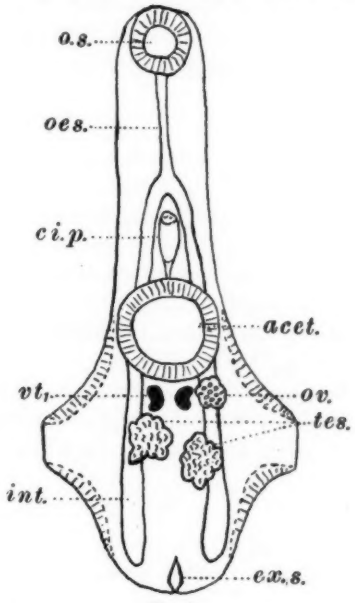

Fig. 3.

Fig. 2. *Catoptroides staffordi* (Pearse). (× 16.) After Pearse.

Fig. 3. *Phyllodistomum angulatum* v. Linstow. (× 36.) *acet.* acetabulum; *ci.p.* cirrus pouch; *ex.s.* excretory sac; *int.* intestine; *oes.* oesophagus; *o.s.* oral sucker; *ov.* ovary; *tes.* testes; vt_1 vitelline gland. After von Linstow.

the uterus; the suckers are of different size, and the neck and oesophagus are much shorter relatively.

The five species described above are the only members of the genus *Catoptroides* Odhner known to science at the present. No two of them occur in the same host and all but one are parasitic in Siluridae; *C. macrocotyle* Lühe is the only one of this group that occurs in Cyprinidae.

Phyllodistomum angulatum v. Linstow (1907) was transferred to the genus *Catoptroides* Odhner by Lühe in 1909, but the facts of the characters as they are given by von Linstow in his description and illustration (Fig. 3) of the species do not justify such a change and, therefore, should be restored to

its original genus. It is characterised by the absence of a groove separating the body into two distinct regions, by having the testes roughly contoured and not deeply lobed and by the fact that the vitelline glands are kidney-shaped and not lobular.

The genus *Phyllodistomum* Braun, 1899, is characterised by the fact that the leaf-like body is not sharply divided into two distinct regions by a groove; the testes are irregular but not deeply lobed and one of them lying considerably more posterior than the other; the vitellaria are less lobed than in *Catoptroides* Odhner. In view of these facts it appears that the above species does not fit into the genus *Catoptroides* Odhner, but much rather into the genus *Phyllodistomum* Braun, in which von Linstow first described it.

SUMMARY.

1. Four species had been described to date as belonging to the genus *Catoptroides: C. spatula* and *C. spatulaeforme* from African catfish and *C. macrocotyle* and *C. angulatus* from European fish.

2. *C. lacustri*, a new species described in this paper, was taken from the great fork-tailed catfish, *Ameiurus lacustris*, from the St Croix River, Minnesota.

3. To this list must be added *C. staffordi* of the American catfish, *Ameiurus nebulosus* and *A. melas*, which was described by Pearse in 1924 and placed in the genus *Phyllodistomum*.

4. *C. angulatus* is shown not to be a member of this genus, but rather as belonging to the genus *Phyllodistomum* in which von Linstow first described it.

5. All of these species are briefly reviewed and compared in this paper.

REFERENCE.

LINSTOW, OTTO F. B. v. (1907). Zwei neue Distomum aus *Lucioperca sandra* der Volga. *Ann. Mus. Zool. L'Acad. Imp. Sci., St Pétersbourg*, **12**, 201–202, 1 fig.

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NEMATODE PARASITES OF VERTEBRATES IN THE PHILADELPHIA ZOOLOGICAL GARDEN AND VICINITY. I.

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(With Plates IV–VIII.)

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I. Introduction.

The following account reports the determinations, with descriptions of new species and morphological data on many others, of the collection of roundworm parasites occurring in animals in the Philadelphia Zoological Garden. Nematodes are by far the commonest parasites, occurring about three times as frequently as all other forms combined; next are the Cestodes, the flukes, and Acanthocephali in the order named. It is from a representative part of the nematode material gathered during the years 1914–1928 that this study was made. The remaining parasites will be reported upon in other papers to follow.

Since in Gardens the heaviest mortality occurs among recent arrivals (Fox, 1923) it is reasonable to assume that the greater number of parasites taken at autopsy have had their origin in the regions whence the hosts came.

Many adult parasites come from hosts which had been too recently captured for the worms to have developed in captivity. Evidence of this is frequent, as witness the fact that common opossums rarely live more than three months

in captivity; nevertheless one will nearly always find mature *Physaloptera turgida* Rudolphi, 1819 present. An opossum in the garden only one day had mature Nematodes. Again, thirteen wild cats received from South Carolina within a few days after their capture were infested with intestinal, muscular, and pulmonary parasites. Dr Strong's recent expedition to Africa proved the presence of a species of *Murshidia* in the wild elephant. A specimen of the same genus was taken from a three-year-old Indian elephant that died in the Garden.

However, some forms are always present in the Garden, as for instance *Haemonchus*, *Trichuris*, and certain Ascarids of universal occurrence, that have been taken from an aoudad (*Ovis tragelaphus*), an American grey wolf (*Canis mexicanus*), a dingo dog (*Canis dingo*), and a green monkey (*Cercopithecus callitrichus*) all born in the Garden. Again the same parasite may be found in several hosts that come from far removed parts of the world. An example is a *Trichuris* from a Bactrian camel (*Camelus bactrianus*) from Central Asia and one from a Rhodesian baboon (*Papio rhodesiae*) from South Africa that have been in captivity only a few months. Similar examples can be given for *Haemonchus* and many Ascarids.

The data are arranged to show the frequency of nematode parasites in a certain host, the susceptibility of certain animals to more than one parasite at the same time, and the infestation of dissimilar hosts by the same parasites. This is brought out in the body of this report, the principal object of which is, however, to make a systematic study of the parasites concerned, listing them accurately according to their present taxonomic position.

The value of such a synoptical list lies in the aid that it gives parasitologists in determining the range of occurrence of parasites, the variety of hosts in which they have been met, and their habitat in the host. The common parasites examined have been named without other comment than the numbers of record and a statement of their habitat and host. Wherever a parasite is found to be new to science, morphological and other data necessary for the identification of the parasite, for the determination of its life-history, and so forth, are given. In the case of some of the rarer and less easily identified forms, short descriptions are given, together with some measurements and figures, partly to supplement previous accounts, but chiefly in order that others may judge the accuracy of the determinations. Many were checked against known material in the Helminthological Collection of the Department of Agriculture in Washington, and in addition, when circumstances warranted, type species were sent for.

At the end of this report are references providing a specific citation of authority for every species named in the list.

II. Acknowledgement.

All of the Zoological Garden worms were placed at the writer's disposal by Dr Herbert Fox, in charge of the laboratory there, to whom I acknowledge my indebtedness. The work was carried out in the Zoological Laboratory of

the University of Pennsylvania under Prof. J. P. Moore. I am very thankful to him for his advice and criticism during the progress of it. Thanks are also due to Prof. C. E. McClung, Director of the Zoological Laboratory, for the use of its facilities.

III. TECHNIQUE AND METHODS OF PROCEDURE.

(*a*) *Method.*

The usual technique was followed, with a few additions by the author, the essentials of which are given here. The Nematodes were taken from 3 per cent. formol, in which they were stored, and placed directly in Carnoy-phenol (Hetherington, 1923). Smaller worms were cleared almost instantly. If the larger specimens did not clear sufficiently at first, almost any degree of clearing was subsequently obtained by allowing the fluid in which they were placed to evaporate, the degree of clearing being proportional to the amount of evaporation. When even greater transparency was desired glacial acetic acid and phenol were added. Because of the tractability of whole worms some measurements were made at this stage.

Since much greater latitude for observation was obtained by utilising degrees of clearing rather than staining, the latter was not employed. Except for low-power work with the microscope *in toto* staining is of little value for Nematodes because of its marked tendency to mask the finer details of structure.

Permanent glycerine jelly mounts (asphaltum varnished) were prepared with only one intervening step, the replacement of the phenol reagent by pure glycerine. It is important not to hurry this process, because the glycerine penetrates to the interior slowly. A gap not too large (5, 10, 20, and 50 per cent.) between the percentages of glycerine and sufficient time for infiltration is important. Allowing evaporation of the alcohol before addition of the next highest percentage glycerine is helpful. Further, it has been found of advantage to mix an equal amount of Carnoy-phenol, itself water free, in the lower percentages of glycerine. This has the effect of keeping the worms dehydrated and clear until thoroughly impregnated with glycerine, when the latter is sufficient. The worm may be manipulated into the desired position on the slide before the warm glycerine jelly becomes cold and set. Cutting off the anterior end in order to get a face view is readily accomplished on the slide after stiffening of the jelly.

(*b*) *Measurements.*

The length of the largest, and in some cases the smallest, that are obviously adults, together with their maximum widths and other measurements are given in millimetres, with a few exceptions which are in micra. Measurements were projected by the camera ludica.

The keys to the genera in Yorke and Maplestone's *The Nematode Parasites of Vertebrates*, 1926, were indispensable for the prosecution of this study.

IV. Incidence of parasites in animal groups, 1903–1923.

The Garden has accumulated records of over nine thousand autopsies performed over a period of twenty-five years. From the standpoint of the host there were frequent and important infestations during that time. The following table (I) shows the percentage incidence of *nematode* parasitism of the bird and mammal hosts for the twenty years 1903–1923. Any hosts family not recorded in the table indicates that there were no important nematode infestations among its members. The "remarks" column shows the most prevalent parasitic genera. This table has been adapted and revised from Fox (1923, p. 630).

Table I.

Incidence of Nematodes in Animal Groups 1903–1923.

Animal	No. autopsied	No. infested	%	Remarks
MAMMALIA				
Primates	538	51	9·4	
Cercopithecidae				
Sooty mangabey *Cercocebus fuliginosus*	34	4	11·8	
Rhesus macaque *Macacus rhesus*	60	6	10·0	
Callitrichidae				
Marmosets *Callithrix jacchus, C. pencillatus*	43	7	16·3	
Cebidae				
Squirrel monkeys *Chrysothrix sciurea*	8	3	37·5	
Other cebus monkeys	87	10	11·5	8 had *Dipetalonema gracilis*
Lemurs	86	6	7·0	
Carnivora	498	84	16·9	
Felidae				
American wild cat *Felis rufus*	28	11	40·0	
Spotted wild cat *F. rufus texensis*	5	4	80·0	
Canada lynx *F. canadensis*	10	4	40·0	Ascarids
Lion *F. leo*	10	3	30·0	„
Ocelot *F. pardalis*	15	5	33·0	*Ancylostoma*
Canidae				
Grey fox *Canis cinerea argenteus*	28	1	4·0	*Ancylostoma*
Red fox *C. vulpes pennsylvanicus*	17	2	12·0	„
Swift fox *C. velox*	5	2	40·0	„
Grey wolf *C. mexicanus*	18	2	11·0	Ascarids
Mustelidae				
American badger *Taxidea taxus*	17	7	41·0	*Physaloptera*
Procyonidae				
Raccoon *Procyon lotor*	42	2	5·0	
Ursidae				
Bears *Ursus tibetanus, U. maritimus, U. malayanus, U. beringiana*	37	6	16·0	Ascarids
Otariidae				
Hair seal *Zalophus californicanus*	20	1	5·0	*Ancylostoma*
Rodentia	198	32	16·0	
Sciuridae				
Squirrels and prairie dogs *Sciurus hudsonicus, Cynomys ludovicianus*	44	4	9·0	
Castoridae				
American beaver *Castor canadensis*	17	4	23·0	*Oxyuris*

Table I (*continued*).

Animal	No. autopsied	No. infested	%	Remarks
MAMMALIA (*cont.*)				
Rodentia				
Hystricidae				
Canada porcupine *Erethizon dorsatus*	47	17	36·0	*Oxyuris; Dirofilaria*
Ungulata	365	44	12·0	
Equidae				
Zebras *Equus zebra, E. burchelli*	7	7	100·0	
Cervidae				
Axis deer *Cervus axis*	6	0	—	
Barasingha deer *C. duvanceli*	8	0	—	
Eld's deer *C. eldi*	6	0	—	
Fallow deer *C. dama*	29	0	—	
Hog deer *C. porcinus*	21	0	—	
Japanese sika deer *C. sika typicus*	14	0	—	
Red deer *C. elaphus*	14	0	—	
Elk *C. canadensis*	29	2	7·0	*Trichuris*
White-tailed deer *Mazama virginiana* ...	33	0	—	
Mule deer *M. hemionus*	8	0	—	
Camelidae				
Llama *Lama glama*	14	2	14·0	
Camels *Camelus bactrianus, C. dromedarius* ...	9	4	44·0	
Suidae				
Wart hog *Phacochoerus aethiopicus*	19	2	10·0	
Edentata	16	2	12·5	
Armadillos *Dasypus sexcinctus*	10	2	20·0	
Marsupialia	175	45	26·0	
Didelphidae				
Common opossum *Didelphys virginiana* ...	84	40	48·0	*Physaloptera; Oxyuris*
Macropodidae				
Kangaroos and wallabies *Petrogale xanthopus, Dorcopsis luctuosa* ...	70	0	—	
AVES				
Passeres				
Corvidae				
Common crow *Corvus brachyrhynchos*... ...	16	7	44·0	*Syngamus; Tetramere*
Magpies *Pica rustica*	28	18	64·0	*Filaria; Tetramere*
Jays *Cyanocitta xanthura*	41	22	55·0	*Filaria; Strongylus*
Pies, choughs, etc.	35	12	33·0	
Sturnidae				
Starlings *Sturnus vulgaris*	63	19	30·0	*Filaria* s.l.
Turdidae				
Thrushes, robins, *Turdus varius, T. musicus, T. viscivorus, T. migratorius*	25	8	33·0	*Filiaria;* 1 robin had *Filaria*
Fringillidae				
Canaries, finches *Serinus canarius, Pipilo, Carpodacus, Carduelis elegans*	24	0	—	
Picariae				
Picidae				
Woodpeckers *Dryotomus pileatus*	4	2	50·0	
Rhamphastidae				
Toucans *Rhamphastus carinatus, Pteroglossus torquatus*	30	9	30·0	*Habronema* or *Acuaria*

Table I (*continued*).

Animal	No. autopsied	No. infested	%	Remarks
AVES (*cont.*)				
Striges				
Owls *Strix, Asio*	142	2	1·4	
Psittaci	774	124	16·0	
Loriidae				
Lorys *Lorius, Eclectus, Trichoglossus*	24	5	20·0	*Habronema* or *Acuaria* (3)
Cacatuidae				
Cockatoos *Cacatua roseicapilla, Calyptorhynchus*	34	2	6·0	*Habronema* (2)
Crested ground parrakeet *Calopsittacus novae hollandiae*	45	4	9·0	„ (4)
Psittacidae				
OLD WORLD (totals)	453	65	14·3	
Undulated grass parrakeet *Melopsittacus undulatus*	121	2	1·6	*Habronema*
Pennant's parrakeet *Platycercus elegans* ...	21	6	29·0	„ (6)
Roselle parrakeet *Platycercus eximius* ...	48	21	44·0	„ (20)
Other old world parrakeets, *Palaeornis, Psittacus*	86	12	14·0	„ (12)
Old World parrots, love-birds, eclectus, *Agapornis*	74	13	18·0	„ (13)
NEW WORLD (totals)	321	69	21·5	
Macaws *Ara macao, A. chloroptera*	26	9	34·0	„ (9)
Conures *Conurus carolinensis*	62	16	26·0	„ (15)
Amazons *Chrysotis aestiva*	164	27	16·5	„ (24)
Other New World parrots	69	17	24·0	„ (7)
Accipitres	201	13	6·7	
Falconidae				
Buzzards *Archibuteo lagopus, Buteo vulgaris* ...	55	4	7·3	
Eagles *Aquila audax, A. clanga*	44	1	2·3	
Vulturidae				
Vultures *Gyps fulvus, G. himalayensis, Vultur monachus*	29	1	3·4	
Other Accipitres and Cathartae *Sarcorhamphus gryphus, Pseudogryphus californianus* ...	73	7	9·6	
Galli	299	42	14·0	
Phasianidae				
Pheasants *Phasianus reevesi, P. torquatus, P. elegans*	95	20	21·0	*Heterakis*
Partridges *Perdix cinerea*	14	3	21·0	„
Quail *Colinus virginianus, Callipepla squamata*	70	10	14·0	„
Wild turkeys *Meleagris gallipavo, M. ocellata*	39	7	18·0	
Columbae	163	14	9·0	
Fulicariae	38	7	18·0	
Alectorides	41	10	25·0	
Gaviae	21	3	14·0	
Steganopodes	22	1	5·0	
Herodiones	105	21	20·0	
Anseres	319	28	8·8	
Swans *Cygnus columbianus, C. buccinator* ...	48	7	14·0	
Geese *Anser cinereus, Bernicla canadensis* ...	83	13	15·6	
Ducks *Anas boscas, A. obscura, A. maculosa*	188	8	4·0	
Struthiones *Struthio camelus*	36	1	2·8	

The table is largely self-explanatory. Some points are significant: for instance, the lack of any nematode parasites in the Cervidae with one exception, the Macropodidae and in the Fringillidae. These were entered in the table to emphasise this singular fact. Other features are the high percentage infestation of squirrel monkeys and marmosets among the Primates; of the wild cats, badgers, and swift foxes among the Carnivora; and of the zebras and camels in the Ungulata. The magpies, jays, woodpeckers, and the common crow lead the Birds. An enzoötic of *Habronema incerta* (Smith, 1908) during 1906–1913 caused an increase in infestation in parrots above the normal 10 per cent. Of all parrots autopsied in each of the above years 25–50 per cent. had this parasite. There were 774 parrots autopsied in the twenty years mentioned with a total incidence of 21 per cent.

V. Significant cases of parasitism.

(*a*) 1923–1928.

In the fiscal year ending March 1, 1924, there were 45 cases of animal parasitism in 366 animals examined. That number of cases gave an incidence of infestation of 12·5 per cent. of all animals autopsied, which was the same percentage incidence as for the previous year. These percentage incidence figures would more exactly express the situation if parasites were definitely searched for. In most cases the attendants who perform the autopsies in the Garden are more interested in finding the cause of death than in finding any parasites. Hence, Nematodes very often escape notice. In addition, many Reptiles and Bactrachians are not autopsied.

As was shown in Table I, nematode parasites had not been observed in wallabies and kangaroos in the Garden. In 1924, however, a yellow-footed rock kangaroo (*Petrogale xanthopus*) yielded six filariform worms up to 12 cm. in length.

During the year 1925 there were no pronounced cases of infestation. The 53 examples of parasitism in the 411 animals examined approximated 13 per cent. Of the six prairie dogs autopsied, five showed liver infestation by *Hepaticola hepatica* (Bancroft, 1893) ova, but no adults were recovered. *Filaria* s.l. occurred eleven times in weavers, finches, and thrushes from two neighbouring cages in the bird house, showing a local distribution and contamination. Five of the twelve common opossums showed Physaloptera.

The year 1926 was without any incident to warrant comment. Forty-nine cases of parasitism in 406 deaths gave a discovery incidence of infestation of 12 per cent.

In the last two years, however, there has been a marked increase of parasitism recorded in the Garden. In the fiscal year ending March 1, 1927, 15 per cent. of all animals autopsied revealed animal parasites—63 out of 424. For the year 1928 the figure is the highest recorded in the history of the Garden, namely 17·6 per cent.—64 out of 364. There have been warnings of a definite increase in the last few years when the figures mounted from the

normal average of around 10 to 12 and 13 per cent. According to Dr Fox this is not entirely due to more careful search since the same procedure as in former years is carried out. The wave may have reached its maximum, but there is no way of telling whether the therapeutic and hygienic measures taken by Drs Fox (1928) and Weidman will be effective or not until another year.

Among the significant cases of parasitism recorded lately, infestation with *Heterakis gallinae* Gmelin in pheasants is noteworthy for frequency. Usually it is limited to two species—Amherst's and golden. This was true in 1927. In 1928 there was a large pheasant mortality (fourteen specimens), 35 per cent. of which were infested. As usual, Amherst's pheasant (*Chrysolophus amhersti*) and the golden pheasant (*C. pictus*) predominated, while a Reeve's pheasant (*Phasianus reevesi*) and a ring-necked pheasant (*P. torquatus*) represented species not commonly infested. Nearly all of the *Heterakis* in quail likewise occurred in one species—scaled quail (*Callipepla squamata*).

At one time *Habronema incerta* (Smith) were present in 25–50 per cent. of the parrots. Fortunately, this enzoötic has passed. The Psittaci contain as a rule few parasites. However, *Habronema* is still a factor in them. In the Cervidae, previously mentioned as noticeably free from parasitism by roundworms, were found during 1927 three cases of whipworm (*Trichuris ovis* (Abildg. 1795)): two in fallow deer, and one in the black-tailed deer. In the Bovidae also were three cases of *T. ovis*: one in the beatrix antelope, and two in aoudads. In 1928 there were nine instances of this infestation in Artiodactyla, spread among two musk oxen, aoudad, kudus, Indian antelope, elk, black-tailed deer and two camels. The two aoudads in 1927 harboured both whipworms and stomach worms (*Haemonchus contortus* Rudolphi, 1803), and of the nine cases exhibiting the former in 1928, seven also harboured the latter. This indicates a definitely increased dissemination.

In the fiscal year ending March 1, 1927, were lost three aoudads (*Ovis tragelaphus*) and an Indian antelope (*Antelope cervicapra*) with the disease caused by this same familiar stomach worm. Examinations at that time showed that all four of the remaining Indian antelopes carried the parasite. Although previously confined to the above hosts, in 1928 the list of hosts was extended to include the buffalo, musk ox, elk and camel—a definite outbreak of stomach worms in the Ungulates. All four of the camels dying in the latter year, both of the musk oxen, and the three Indian antelopes were infested—eleven cases in all. This parasite is to be regarded as established in the Garden as long as coprophagous rats have access to the enclosures. Both whipworm and stomach worm ova have been taken from the caeca of Norwegian wild rats (*Mus norvegicus*), killed in the Garden and sent to the writer for observation.

The familiar *Physaloptera turgida* was met five times in the common opossum (*Didelphys virginiana*) in 1927. In 1928 the common opossums lived up to their unsavoury reputation. Out of the five autopsied, four exhibited *Physaloptera* in the stomach and the other an oxyurid in the small intestine.

Five Derby's opossums (*D. derbianus*) were acquired this year. They died within three months, but none exhibited *Physaloptera*.

Ascarids appear to be fairly well controlled at this time, evidently due to the santonin treatment employed in practice many years. Hookworm likewise is not especially frequent.

Table II.

Summary percentage incidence of animal parasitism 1923–1928.

Fiscal year ending Mar. 1	Animals exhibited	Total autopsies	Mammalia	Aves	Reptilia	Amphibia	Pisces	Cases of parasitism	% incidence of infestation
1924	1871	366	95	258	11	1	1	45	12·0
1925	1924	411	100	291	19	—	1	53	13·0
1926	1928	406	118	281	6	—	1	49	12·0
1927	1877	424	143	276	5	—	—	63	15·0
1928	1806	364	98	262	4	—	—	64	17·6

(*b*) *Superparasitism.*

Superparasitism has occurred many times in the Garden. Three of the hosts mentioned in this account were parasitised with three different species of Nematodes at one time. In the following table will be seen a list of those that harboured more than one species of roundworm when autopsied.

Table III.

Occurrence of superparasitism.

	Host	Parasite
8618 and 9015	*Rhea americana* (S. Am. Rhea)	*Dicheilonema rheae* (Owen, 1843) *Habronema incerta* Smith, 1908
3481	*Colinus virginianus texanus* (Texas bobwhite quail)	*Cyrnea colini* Cram, 1927 *Heterakis gallinae* (Gmelin, 1790) *Subulura brumpti* (Lopez-Neyra, 1922)
4245 and 4819	*Didelphys virginiana* (Common opossum)	*Physaloptera turgida* Rudolphi, 1819 *Cruzia tentaculata* (Rudolphi, 1819)
3293	*Felis rufus texensis* (Spotted wild cat)	*Ancylostoma caninum* (Ercolani, 1859) *Toxascaris leonina* (Linstow, 1902)
3221	*Canis velox* (Swift fox)	*Ancylostoma caninum* (Ercolani, 1859) *Toxocara canis* (Werner, 1742)
8464	*Ursus maritimus* (Polar bear)	*Haemonchus contortus* (Rudolphi, 1803) *Toxascaris transfuga* (Rudolphi, 1819) *Trichinella spiralis* (Owen, 1835)
6190	*Erethizon dorsatus dorsatus* (Canadian porcupine)	*Dirofilaria spinosa* sp. nov. *Dipetalonema diacantha* (Molin, 1858)
5741	*Papio hamadryas* (Arabian baboon)	*Physaloptera caucasica* Linstow, 1902 *Subulura distans* (Rudolphi, 1809) *Trichuris trichiura* (Linnaeus, 1771)
5043	*Papio hamadryas* (Arabian baboon)	*Subulura distans* (Rudolphi, 1809) *Trichuris trichiura* (Linnaeus, 1771)
7113	*Papio rhodesiae* (Rhodesian baboon)	*Subulura distans* (Rudolphi, 1809) *Trichuris trichiura* (Linnaeus, 1771)

VI. Distribution.

(*a*) *Location within the host.*

The organ most commonly parasitised is the intestine. Nematodes are most numerous in the small intestine, then in the caecum, then in the colon. The stomach ranks second; the proventriculus of birds, in this respect, corresponding to the stomach of Mammals. The peritoneum ranks third, with mesenteric parasites fourth.

One filariform worm was found in three locations in one host, the yellow-footed rock kangaroo mentioned previously. Two lay free in the stomach, two were in the peritoneal cavity with both ends embedded in the liver, and one had quite penetrated the diaphragm, with as much of the body in the pleural cavity as in the peritoneal. This is a most striking example of the power of parasites to migrate through solid tissue.

There were more females present than males in any group. Where but one or a few only occurred, they were usually females.

(*b*) *Geographical locality.*

The locality whence the host came is indicated in the synoptic list. Hosts born in the Garden are thus mentioned. The reader may judge from the time the host has spent in the Garden whether infestation originated in the wild or not. It is for this reason that the length of their stay in the Garden is indicated.

VII. Synoptic list, including new species.

This list of nematode parasites, comprising Part I of a series based upon collections made during the years 1914–1928, is arranged according to families in the order given by Yorke and Maplestone (1926). Some morphological data accompany every name of a Nematode in the list. Only a few facts are given with the common forms *Ascaria* and *Haemonchus*. But many in this list are rare and seven are new to science. Hence, fuller descriptions of them are included. The new species are described under their families in their proper taxonomic position.

The P.Z.G. (Philadelphia Zoological Garden) numbers and host names following each species listed indicates the number of separate determinations that were made.

Family TRICHURIDAE Railliet, 1915.

Genus **Trichuris** Roederer, 1761.

1. *Trichuris ovis* (Abildgaard, 1795).

The whipworms present in the collection fall into two species, *T. ovis* and *T. trichiura*. Of the former adults have been found to vary from 26 to 45 mm. in length in the male and from 29 to 50 mm. in the female; with a diameter in the male of 0·44–0·62 mm. posteriorly and 0·17 mm. anteriorly and in the female of 0·62–0·92 mm. posteriorly and 0·089–0·143 mm. anteriorly. The spicule is 28–40 micra wide and covered by a spinous sheath 90 micra wide.

In length it is 4·92 mm. The ova are 60 micra by 32 micra. Five different hosts are recorded:

P.Z.G. 3855 *Camelus bactrianus* (Bactrian camel). Colon, heavy infestation; Central Asia; 11 yr.
P.Z.G. 4840 *Lama huanacos* (huanaco). Caecum, many present; Peru to Patagonia; 5 yr.
P.Z.G. 4680 *L. glama* (llama). Caecum, heavy infestation; born in the Garden; 1 mo.
P.Z.G. 6435 *Ovis vignei* (Punjab wild sheep). Colon, many present; N.W. India; 4 yr.
P.Z.G. 8887 (b) *Ovibos wardi* (White-fronted musk ox). Colon, heavy infestation; North Greenland and Grant Land; —.

2. *Trichuris trichiura* (Linnaeus, 1771).

This species, which is found in man, was met only in Primates in the Garden. In three of the hosts it was met in company of individuals of the genus *Subulura* Molin. Males are 30·6–33 mm. and females 31–35 mm. long. The former has a fairly constant breadth posteriorly of 0·52–0·54 mm., the latter of 0·64–0·71 mm. The spicule varies from 2·15 to 2·72 mm. in length. The ova in ovijector measure 50–55 × 20–23 micra. In every case females were more numerous than males, and in the material from two hosts no males were seen.

P.Z.G. 5043 (b) *Papio hamadryas* (Arabian baboon). Caecum, many females, no males; Arabia and N.E. Africa; 10 yr.
P.Z.G. 5741 (c) *P. hamadryas* (Arabian baboon). Caecum, heavy infestation; 2 yr. 2 mo.
P.Z.G. 7113 (a) *P. rhodesiae* (Rhodesian baboon). Colon, 1 male, 2 females; Umtali, S. Rhodesia; 2 yr. 8 mo.
P.Z.G. 6479 *Hylobates leuciscus* (silver gibbon). Colon, many females, no males; Java; 12 days.

Family STRONGYLIDAE Baird, 1853.

Genus **Poteriostomum** Quiel, 1919.

3. *Poteriostomum imparidentatum* Quiel, 1919.

This genus was split up by Quiel in 1919 and again by Kotlán (1919) who gives a key separating *imparidentatum* from *pluridentatum* Quiel, 1919, on the basis of seven and ten elements respectively between the larger ones in the leaf crown. This lot from one host shows both conditions. It is erroneous to state that there are two species because of this difference, as otherwise they agree. The writer confirms Yorke and Maplestone's listing of the latter as a synonym of the former, believing this to be within the range of variation for this species. Unfortunately, Yorke and Maplestone (p. 60) copy Yorke and Macfie's incorrect illustration of the ventral cleft ray which shows it arising from a common base with the external-lateral and laterals in the tail of the male. It is distinctly separate and more median (Pl. IV, fig. 3). The males are 10 × 0·58 mm.; the females 11 × 0·603 mm. in length by breadth. The spicules, which slide against a gubernaculum, are equal, slender, and 1·34 mm. long. The nerve ring is 0·4 mm. from the anterior extremity and the oesophageal constriction is 0·85 mm. from the same. The vulva lies 9·7 mm. from the anterior

end of the body. The anal pore is just midway between the vulva and the posterior end. The buccal capsule measured 0·14 × 0·16 mm.

P.Z.G. 8459 *Equus zebra* (mountain zebra). Colon, congested by heavy infestation; S. Africa; 11 yr. 8 mo.

Genus **Murshidia** Lane, 1914.

4. *Murshidia falcifera* (Cobbold, 1882).

Three species of this genus are recorded from the Indian elephant. According to Khalil (1922, p. 205) nematode parasites of elephants are peculiar to each species.

Bursa ray formula and other characters of this material are as in the type. The males are smaller than the type, 19·3 and 20·5 × 0·82 mm.; but the females are of the same size, 23 and 27 × 0·84 mm. The stout male oesophagus is 0·9 mm. long by 0·38 mm. in width. The buccal capsule is 0·06 mm. deep with an inside diameter of 0·14 mm. The vulva is 24·28 mm. from the anterior end and 0·56 mm. in front of the anus. The hockey-stick-shaped spicules measure 1·58 mm., slightly greater than in the type. The leaf crown arising from the internal surface of the buccal capsule has eighty rays. A figure of the bursa of this rare species is given (Pl. IV, fig. 2). The host, a young male Asiatic elephant, was received in the Garden from the Jambi District, Sumatra, and was supposed to be about three years old when it died.

P.Z.G. 5005 *Elephas maximus* (young Asiatic elephant). Intestine, many; Sumatra; 3 mo.

Genus **Oesophagostomum** Molin, 1861.

5. *Oesophagostomum venulosum* (Rudolphi, 1809).

The outstanding character that undoubtedly places this species is the presence of sixteen rounded external oral leaf elements. The species nearest to this is *columbianum* Curtice, 1890, with twenty-four. Attempts of Railliet and Henry (1913) and others to divide this genus into four subgenera are undesirable and unnecessary at this time. The writer agrees with Thornton (1924, p. 395) who quotes Goody (1924) that when a subgenus of this genus contains but a single species it is untenable. The morphological differences are too small. Ransom (1911, p. 46) says there are sixteen internal leaflets; Thornton (1924, p. 395) says twice as many as external. These have the same number, sixteen internal. The males measure 11 × 0·44 mm.; the females 12 × 0·528 mm. The spicules are slender, equal, fused, and 1·37 mm. long. The nerve ring is 0·33 mm. from the anterior end, and the ventral transverse groove 0·28 mm. The cuticular flanges and a gubernaculum are both present, and the oesophagus is 0·85 mm. in length, having a buccal capsule with an inside diameter of 0·07 mm. The vulva is 11·6 mm. from the anterior end. The anus of the female is 0·17 mm. from the posterior end and 0·23 mm. posterior to the vulva. This adds a new host and new locality record for the species.

P.Z.G. 6418 *Cervus cashmirianus* (Kashmir deer). Colon, heavy infestation; Valley of Kashmir; 3 yr. 4 mo.

Family ANCYLOSTOMIDAE (Looss, 1905) Lane, 1917.

Genus **Ancylostoma** (Dubini, 1843) Creplin, 1845.

6. *Ancylostoma caninum* (Ercolani, 1859).

The common hookworm of carnivores was met four times. Twice it was associated with ascarid forms of the genera *Toxascaris* and *Toxocara*. The males are 7·77–8·9 mm. in length by 0·36 mm. in breadth, the females 9·1–12 mm. by 0·38–0·46 mm. The buccal capsule with the characteristic three pairs of hooks, the size of the spicules, equal, slender, and 0·82 mm. long, and the bursal formula limit these to *caninum*. The vulva is 7·95 mm. from the anterior end. The anal orifice of the female lies 0·17 mm. from the posterior end.

P.Z.G. 3947 *Felis pardalis* (ocelot). Intestine, one present; Texas and Mexico; 2 mo.
P.Z.G. 3298 *F. rufus texensis* (spotted wild cat). Intestine, a few only; Texas to California; 2 mo.
P.Z.G. 3293 (a) *F. rufus texensis* (spotted wild cat). Intestine, a few only; 2 mo.
P.Z.G. 3221 (a) *Canis velox* (swift fox). Intestine, 4 present; Western U.S.; 10 yr.

7. *Ancylostoma pluridentatum* (Alessandrini, 1905).

A comparatively rare hookworm that has been mentioned in the literature not more than five times heretofore. The heavy ventral two-toothed cutting organs and the three pairs of dorsal smaller teeth form a circlet around the mouth (Pl. IV, fig. 1). These are clearly defined specific characters. The male measures 6·5 × 0·28 mm.; the female 8·5 × 0·31 mm. Just posterior to the nerve ring, which is 0·43 mm. from the anterior end, are two large papillae directed caudad. The club-shaped oesophagus is 0·68 mm. long and the included buccal capsule 0·14 mm. long. The typical gubernaculum measures 0·06 × 0·01 mm. This form has been found in *Felis onça*, *F. tigrinus*, and *F. eyra* (see Schwartz, 1927). The host of this material is unknown, due to an error in the autopsy record.

Genus **Brachyclonus** Railliet and Henry, 1910.

8. *Brachyclonus indicus* Railliet and Henry, 1910.

This hookworm, the only species placed in the genus, has never been found in any other host than that from which I report it, and that only rarely. The male is 11 × 0·59 mm. and the female 11 × 0·6 mm. The spicules are equal but there is no gubernaculum. Dorsal ray single, forked distally. Trunk long. Each branch bidigitate as shown by Khalil (1922). Closer examination of each branch reveals that it is tridigitate. The most median branch is divided at the end (Pl. IV, fig. 4). Khalil's illustration of the mouth shows a continuous oral margin. There are pulp-like denticles on the dorsal and lateral margins acting as supports as in the genus *Gaigeria* Railliet and Henry, 1910. The illustrations (Pl. VIII, figs. 44, 45) are reproduced to correct these errors and to add to our knowledge of the species.

P.Z.G. 6824 *Tapirus indicus* (Malayan tapir). Intestine, few only; Malacca, Sumatra and Borneo; 1 mo.

Genus **Monodontella** Yorke and Maplestone, 1926.

9. *Monodontella giraffae* Yorke and Maplestone, 1926.

Another hookworm that is the only species placed in the genus. This is the first time it has been found since the type was described from material in England. Dr W. Yorke, Liverpool, kindly sent the type specimens for comparison and mine proved identical. The asymmetrical bursa formula is exactly as in the type. A slightly different view of the bursa is illustrated (Pl. IV, fig. 5). The males are 14 × 0·43 mm., and females 19·1 × 0·66 mm. The buccal capsule is directed dorsally. The club-shaped oesophagus is 1·58 mm. long. The fused spicules with barbed tips are 0·96 mm. long. The anal orifice of the female is 0·35 mm. from the caudal end, whereas the vulva is just in front of the middle.

P.Z.G. 4209 *Giraffa camelopardalis* (giraffe). Bile duct, few only; Africa; 5 yr. 9 mo.

Family TRICHOSTRONGYLIDAE Leiper, 1912.

Genus **Haemonchus** Cobb, 1898.

10. *Haemonchus contortus* (Rudolphi, 1803).

This common stomach worm is often found in ruminants. The males are 9–23 mm. in length by 0·19–0·27 mm. in width, the females 18–27 mm. by 0·33–0·42 mm. The prominent vulvular flap, asymmetrical dorsal lobe of the bursa, and the lancet tooth fix this species readily.

P.Z.G. 8924 *Camelus dromedarius* (common camel). Abomasum; Arabia, Egypt; 1 yr.
P.Z.G. 8565 *Antelope cervicapra* (Indian antelope). Stomach, heavy infestation; India; —.
P.Z.G. 8541 *Ovis tragelaphus* (aoudad). Stomach and caecum, very heavy infestation; N. Africa; —.
P.Z.G. 8458 *O. tragelaphus* (aoudad). Pylorus, many present; N. Africa; 3 mo.
P.Z.G. 6852 *O. tragelaphus* (aoudad). Stomach, many present; born in Garden; 4 mo.
P.Z.G. 6363 *O. tragelaphus* (aoudad). Bolus in pyloric valve, heavy infestation; born in Garden; 3 mo.
P.Z.G. 6367 *O. tragelaphus* (aoudad). Stomach and caecum, heavy infestation; born in Garden; 3 mo.
P.Z.G. —— *Ovibos moschatus* (musk ox). Stomach; Northern Barrens of N. America; —.
P.Z.G. 8887 (a) *O. wardi* (white-fronted musk ox). Abomasum, very heavy infestation; N. Greenland and Grant Land; —.
P.Z.G. —— *Bison americanus* (American bison, "buffalo"). Stomach; N. America; —.
P.Z.G. 8464 (a) *Ursus maritimus* (polar bear). Duodenum many, few in large intestine; Arctic regions; 19 yr. 8 mo.

Genus **Ostertagia** Ransom, 1907.

11. *Ostertagia mentulata* Railliet and Henry, 1909.

A bursated parasite in the same host from which Railliet and Henry (1909) described their type. It has the typical broad spicules, 0·69–0·71 mm. in length. The winged tip has its longer process jointed and movable at the base, and it has a membranous inner part. The small dorsal accessory bursal membrane, supported by two rays, the prebursal papillae, and the external dorsal

ray arising separately from the dorsal, are characters of *mentulata*. The male is 6–6·5 × 0·11 mm., and the female 8–10 × 0·13 mm. The vulva is 8·09 mm. from the anterior end. The anal orifice of the female is 0·10 mm. from the caudal end. Ova are in cleavage stages at time of deposition. They are relatively very large (70–80 × 35 micra) and few in number.

P.Z.G. —— *Camelus bactrianus* (Bactrian camel). Intestine; India; —.

Genus **Ornithostrongylus** Travassos, 1914.

12. *Ornithostrongylus quadriradiatus* (Stevenson, 1904).

The inflated cephalic cuticle, the ring-like gubernaculum surrounding the equal spicules, and the presence of pre-bursal papillae place these specimens in *quadriradiatus*. The spicules end in three points and are 0·15 mm. in length. The males are 7·2–8 × 0·06–0·07 mm., the females 12–18 × 0·13 mm. The vulva is 15·12 mm. from the anterior end. The female has a filament in the centre of the blunt posterior end. The anal orifice is 0·14 mm. from this end.

P.Z.G. —— *Columba livia* (pigeon). Intestine, few only; Phila., Pa., U.S.; —.

Genus **Graphidioides** Cameron, 1923.

13. *Graphidioides affinis* (Megnin, 1895).

A red, bursated thread-worm from the stomach of a Patagonian cavy. The bursa ray formula agrees with the type. The spicules are long, equal, double rods, joined at the tips. Male, 17·6 × 0·18 mm.; female, 16·5–21 × 0·23 mm.

P.Z.G. 8562 *Dolichotis patachonica* (Patagonian cavy). Stomach, heavy infestation; Patagonia into Argentina; 7 mo.

Family OXYURIDAE Cobbold, 1864.

Genus **Trypanoxyuris** Vevers, 1923.

14. *Trypanoxyuris trypanuris* Vevers, 1923.

Dr G. M. Vevers wrote that he had not heard of anyone finding this worm since he found it in the intestine of Humboldt's saki in 1921. This was in the forest of Northern Guiana. He collected a single male specimen and many females. It is not surprising that he missed other males since they are so small. His descriptions (1923, pp. 39–40) and his figure of the male caudal end are good, but his figures of the anterior are inaccurate. For such a small worm the two lips are well defined (Pl. VIII, fig. 50). His male is 2 mm. long by 0·15 mm. in diameter, and his females 6·7 mm. by 0·6 mm. These males are 1·58–1·8 mm. long by 0·08–0·1 mm. in diameter, and the females 4·8–5·64 mm. by 0·3–0·37 mm. The species is rare, it lacks good illustrations, and it has greater size range than indicated in the type description.

The oesophagus bulges into a distinct posterior bulb. Three valves lead from it into the intestine. In the female the anterior oesophagus measures 0·8 mm. in length, the bulb 0·13 mm.; the entire oesophagus, therefore, is

0·93 mm. long. The vulva is 1·81 mm. from the anterior end. Females bulge out wide and dark from the many eggs present. The eggs are elliptical and measure 38–41 × 20–21 micra. The female tail is drawn out into a fine point. The two lips of the female are definite and protrude cephalad a little. The pulp is prominent and two very small papillae are present on each lip. There is a large oral papilla in place of interlabia. The female cephalic cuticle is inflated (Pl. VIII, fig. 51). The single spicule with its ringed gubernaculum in the male is stated to be 0·07 mm. long by Vevers. In this it is 0·04–0·05 mm. long. The arrangement of the five pairs of caudal papillae and the presence of a spike at the end of the tail are alike in both (Pl. IV, fig. 9). The spike is 0·02 mm. in length. This is a new host and a new locality for the species. The host came direct to the Garden from Nicaragua.

P.Z.G. 3208 *Ateles geoffroyi* (black-handed spider monkey). Caecum, 138 females, 16 males; Nicaragua; 7 mo.

Family HETERAKIDAE Railliet and Henry, 1914.

Genus **Heterakis** Dujardin, 1845.

15. *Heterakis gallinae* (Gmelin, 1790).

This is the very common parasite of poultry and pheasants. It is the type of the genus. Yorke and Maplestone (1926) state that *H. vesicularis* (Froelich, 1791) is the type. Miss Cram (1927, p. 52) correctly gives *gallinae* as the type on the basis of priority. It is 11·1 mm. in length by 0·38–0·51 mm. in width in the male, and 9·5–13·6 by 0·45–0·54 mm. in the female. Twelve pairs of ventral caudal papillae and a well-developed notched chitinous sucker are present. The left spicule is 1·72–1·92 mm. long. The right spicule is 1·78–1·93 mm. long. There is a bulb on the oesophagus. The sucker has an outside diameter of 0·13–0·15 mm. The vulva is 6·03 mm. from the anterior end. The anal orifice of the female is 1·72 mm. from the pointed tail end.

P.Z.G. 3201 *Chrysolophus pictus* (golden pheasant). Caeca, many; China; 4 yr. 2 mo.
P.Z.G. 3301 *C. pictus* (golden pheasant). Caeca, many; China; 11 mo.
P.Z.G. 3481 (a) *Colinus virginianus texanus* (Texas bobwhite quail). Caeca, many; Texas; 48 days.

16. *Heterakis isolonche* Linstow, 1906.

A Heterakid with the caudal alae crossing anteriorly to the ventral sucker. The spicules are equal. The left is broadly alate and asymmetrical at the tip. The right is smooth and tapers gradually to the point. There are twelve pairs of caudal papillae present. Males, 8–8·5 × 0·44–0·47 mm.; females, 11·2–12·5 × 0·51 mm. The chitinous sucker measures 0·1–0·14 mm. in outside diameter. The lips are without interlabia.

P.Z.G. 8261 *Crossoptilon auritum* (Manchurian eared pheasant). Caecum, many; N.W. China; 4 yr. 8 mo.

Family SUBULURIDAE Yorke and Maplestone, 1926.

Genus **Subulura** Molin, 1860.

17. *Subulura brumpti* (Lopez-Neyra, 1922).

The genus *Subulura* to the writer's knowledge has never been reported from the quail before. The tail formula of ten pairs of papillae and the prolonged tip fits the description of this species. It measures in the male 9 mm. in length by 0·44 mm. in width; in the female 13–14·3 mm. by 0·57 mm. The equal spicules are 1·34 mm. long. The vulva is at the junction of the anterior third of the body. The anus is 1·36 mm. from the tail end.

P.Z.G. 3481 (c) *Colinus virginianus texanus* (Texas bobwhite quail). Proventricle, very many; Texas—S.E. N. Mexico; 48 days.

P.Z.G. 6083 *C. virginianus texanus* (Texas bobwhite quail). Proventricle, very many; Texas —S.E. N. Mexico; 4 yr. 3 mo.

18. *Subulura distans* (Rudolphi, 1809).

Eleven hosts had this species present. The figure of the male tail shows the papillar arrangement (Pl. VI, fig. 26). The smallest males are 9 mm. in a spotted wild cat; the largest are 18·5 mm. in a pigtailed macaque. The smallest females are 14 mm. in the spotted wild cat; the largest are 24 mm. in a hamadryas baboon. The more frequent measurements are 15–16·5 mm. for males and 22–23 mm. for females. Diameter of the body is 0·27–0·62 mm. in males and 0·39–0·87 mm. in females. The males have ten pairs of ventral papillae. The equal spicules are 1·76–2·42 mm. long. The sucker is 0·2–0·24 mm. in diameter. The vulva is 13·3 mm. from the anterior end. The anal pore of the female lies 1·37–1·8 mm. from the tail end. The elliptical ova are 57–65 × 42–45 micra in size. Spotted wild cat is a new host for this species.

P.Z.G. 4537 *Felis rufus texensis* (spotted wild cat). Intestine, about 50; Texas to California; 3 yr. 1 mo.

P.Z.G. 5043 *Papio hamadryas* (Arabian baboon). Caecum; Arabia and N.E. Africa; 10 yr. 1 mo.

P.Z.G. 5741 (b) *P. hamadryas* (Arabian baboon). Caecum, heavy infestation; 26 mo.

P.Z.G. 7113 (b) *P. rhodesiae* (Rhodesian baboon). Colon, few; Umtali, S. Rhodesia; 2 yr. 8 mo.

P.Z.G. 6909 *Macacus inuus* (Barbary ape). Caecum and large intestine, heavy infestation; N. Africa (Gibraltar); 5 yr. 10 mo.

P.Z.G. 9033 *M. pileatus* (toque macaque monkey). Caecum, heavy infestation; Ceylon; 11 yr.

P.Z.G. 4663 *M. nemestrinus* (pigtailed macaque). Intestine, few; Sumatra and Borneo; 10 yr. 5 mo.

P.Z.G. —— *M. rhesus* (common macaque monkey). Passed; India; —.

P.Z.G. 7290 *Cercopithecus callitrichus* (green monkey). Colon, heavy infestation; born in the Garden; 5 yr. 5 mo.

P.Z.G. 6022 *C. callitrichus* (green monkey). Colon, many; West Africa; 3 yr. 6 mo.

P.Z.G. 7227 *C. lalandii* (vervet monkey). Colon, 12 present; Africa; —.

19. *Subulura jacchi* (Marcel, 1857).

The Subuluras show host specificity in marmosets. They possess ten pairs of ventral caudal papillae plus a small one on the cloacal lip. Barreto (1919)

gives eleven pairs. Barreto (1919, p. 24), in writing of the sucker, states: "lower limit 0·71 mm. from caudal extremity," and, "Anus 1·164 from caudal extremity." This is an error as it would put the anus before the sucker. The original description states (p. 29): "limite inferior á 0·719 mm. da extremidade caudal," and, "Anus á 0·169 mm. da extremidade caudal." This is correct.

The males of this lot are constant in size. They are 9·5–10·5 mm. long by 0·43–0·52 mm. wide. The females vary from 14 to 21·3 mm. by 0·41–0·8 mm. The spicules measure: right, 1·64–1·66 mm.; left, 1·61–1·67 mm. The gubernaculum is 0·21–0·24 mm. The sucker is 0·33–0·35 mm. from the cloaca. The cloaca is 0·22–0·24 mm. from the posterior end. The vulva is 7·03 mm. from the anterior end. The anus is 0·84 mm. from the posterior end.

P.Z.G. 4020 *Callithrix jacchus* (common silky marmoset). Caecum, many; Southern Brazil; 11 mo.
P.Z.G. 4030 *C. jacchus* (common silky marmoset). Caecum, heavy infestation; Brazil; 11 mo.
P.Z.G. 5172 *C. pencillatus* (black-eared marmoset). Caecum, heavy infestation; Brazil; 1 yr.
P.Z.G. 5379 *Leontocebus oedipus* (pinche marmoset). Caecum, few; Columbia; 18 mo.
P.Z.G. 5399 *L. oedipus* (pinche marmoset). Caecum, few; Columbia; 19 mo.

20. *Subulura pennula*, new species, Pl. VII, figs. 33, 34, 35, 36; Pl. VIII, figs. 52, 54.

A new species closely related to *Subulura brumpti* (Lopez-Neyra, 1922). The arrangement of the caudal papillae, the dorsally curved head, the cuspidate tail spike and position of the vulva are similar. It differs from *brumpti* as follows: It has double alae upon the tail of the male, larger spicules, a different arrangement of the posterior papillae, a papilla upon the cloacal lip, larger ova, and, finally, cephalic alae which are one-eighth of the body length. In *brumpti* the latter are one-sixth of the body length.

This species has four small teeth at the beginning of the oesophagus. The mouth is without lips. There are six head papillae in two lateral groups of three each. The lateral cephalic alae extend caudad 0·8–0·9 mm., one-half the distance to the oesophageal bulb. Each ala is 40 micra at its broadest part.

Male. The males are 5·5–7·5 mm. in length and 0·31–0·34 mm. in width. The oesophagus bulges out posteriorly and measures 0·85 mm. in length. The bulb is 0·12–0·15 mm. in diameter. The anterior end of the intestine spreads out widely where it joins the bulb. The muscular sucker is 170 micra long. From it to the cloacal aperture is 0·35–0·37 mm. From the cloacal aperture to the caudal end of the body is 0·34–0·36 mm. A cuspidate spike protrudes 115 micra from the end of the tail. The spicules, sub-equal, alike, and prominently channelled, are 1·04 mm. in length on the right of the body, and 0·93 mm. on the left. They slide in a horned gubernaculum 185 micra long. Ten pairs of caudal papillae are present and one additional unpaired papilla upon the cloacal lip. Three pairs are sessile and pre-anal: the most anterior pair is lateral to the sucker; the next pair is 140 micra from the sucker and about a fourth of the distance to the cloaca; the third pair is just anterior to the cloaca. Two pairs are adanal: one pair is lateral and supports the caudal alae; the other pair is

sessile and ventral to this pair. Five pairs are post-anal: the first two pairs are sessile; the third is lateral and supports a well-defined second alae; and two pairs are sessile in a group at the tail end. Caudal glands open just anterior to these last two pairs.

Female. The females are 10·5–12 mm. in length and 0·41–0·51 mm. in diameter, nearly twice the length of the males. The oesophagus is 1·04–1·38 mm. from teeth to bulb. The latter is 0·2–0·22 mm. in diameter. The vulva is slightly salient and 4–4·3 mm. from the head end, dividing the body into a ratio of 1 : 3. The anal orifice is 1·26–1·3 mm. from the end of the finely subulate tail. Mature ova in uteri are elliptical and measure 60 × 38 micra. The genus *Subulura* has never been reported from this host. Type 3436, deposited in the Zoological Laboratory, University of Pennsylvania. Paratypes in P.Z.G.

P.Z.G. 3435 *Callipepla squamata* (scaled quail). Caeca, 12–15 only; Texas to Arizona and Mexico; 16 days.

P.Z.G. 3436 *C. squamata* (scaled quail). Caeca, 12–15 only; Texas to Arizona and Mexico; 16 days.

P.Z.G. 3437 *C. squamata* (scaled quail). Caeca, few only; Texas to Arizona and Mexico; 17 days.

Family KATHLANIIDAE (Travassos, 1918).

Genus **Spironoura** Leidy, 1856.

21. *Spironoura procera*, new species, Pl. VI, figs. 22, 23, 24, 25.

The reptilian parasites belonging to this genus are divided into those having pre-anal muscles well developed into a sucker-like organ and those without. This new one has no pre-anal sucker-like organ; it is thus in the group with *S. gracile*, *S. affine*, *S. congolense*, *S. falcatum*, and *S. testudinis*. It is more closely related to *S. testudinis* (Baylis and Daubney, 1922) than to any of the others. It differs from that species, however, in having ten pairs of caudal papillae. It has one unpaired papilla on the anterior lip of the cloaca also. *S. testudinis* has eleven pairs, and they are arranged differently. The lateral pair is number two from the posterior in *procera* while in *testudinis* it is number three. Five, six, and seven are evenly spaced in *procera*. The tail of both the male and the female is half again as long as in *S. testudinis*.

Male. The males are 13 mm. in length and 0·41 mm. in diameter. The mouth has three lips, each bearing two outer and two inner papillae. The long narrow oesophagus, which is 2·31 mm. long, ends in an hour-glass-shaped posterior bulb. The pharynx measures 0·15 mm. long. The nerve ring is 0·66 mm. from the anterior end. The two equal spicules, 0·97 mm. long by 0·05–0·06 mm. wide, slide in a partly chitinised, flared gubernaculum. The chitinous part of the latter measures 0·18 mm. in length. Anus to posterior end is 1·4 mm. The conical tail is drawn out into a very fine point and curves ventrally. The position of the anus and the shape of the caudal region is the same in both sexes. Ten pairs of papillae are arranged as follows: number one at the tip of the tail is ventral; number two is lateral; number three ventral, but close to and anterior to number two; number four lateral, just over number five; numbers five, six,

and seven in an evenly spaced ventral row, the last just posterior to the cloacal rim. The three evenly spaced anterior pairs and the one unpaired upon the cloacal rim are the same as in all species of the genus.

Female. The female is 15 mm. in length and 0·42 mm. in width. This is slightly greater than the male. The vulva lies in the posterior third and is 4·05 mm. from the anus and 9·65 mm. from the anterior end. The anal orifice is 1·3–1·35 mm. from the sharp tail end. Type deposited in the Zoological Laboratory, University of Pennsylvania. Paratypes in P.Z.G.

P.Z.G. 6662 *Chrysemys rubiventris* (red-bellied terrapin). Large intestine, heavy infestation; Middle Atlantic States; —.

Family CRUZIIDAE (Travassos, 1917).

Genus **Cruzia** Travassos, 1917.

22. *Cruzia tentaculata* (Rudolphi, 1819).

This also is the only species in its genus. These specimens possess a chitinous vestibule 0·18 mm. long armed with rows of curved teeth. They also have an anterior intestinal diverticulum of 1·07 mm. The males are 9 × 0·42 mm., the females 11 × 0·47 mm. The oesophagus has a bulb. The two sub-equal similar spicules are 0·96 and 0·93 mm. long and slide against a spike-like gubernaculum 0·13 mm. in length. The cloaca is 0·17 mm. from the tail end. The anal pore of the female is 0·8 mm. from the tail end, while the vulva is 4·72 mm. from the head end. The caudal papillae have bulbous bases and a different arrangement of numbers two and three from the tail end than in Yorke and Maplestone's original drawing (Pl. IV, fig. 7).

P.Z.G. 4245 *Didelphys virginiana* (common opossum). Colon, few; U.S.; 1 day.
P.Z.G. 4819 *D. virginiana* (common opossum). Small intestine; U.S.; 6 days.

Family ASCARIDAE Baird, 1853.

Genus **Ascaris** Linnaeus, 1758.

23. *Ascaris lumbricoides* Linnaeus, 1758.

The common large *Ascaris* of man, monkey and pig. No interlabia, no alae. The dorsal lip pulp has two fan-like anterior lobes. The males are 122–132·7 × 2–2·5 mm., the females 157–191 × 3·3–4·6 mm.

P.Z.G. 6794 *Alouatta seniculus* (red howler monkey). Stomach, 15; intestine, 36; S. America; 2 mo.
P.Z.G. 6828 *A. seniculus* (red howler monkey). Large intestine, 32; 3 mo.

Genus **Toxocara** Stiles, 1905.

24. *Toxocara mystax* (Zeder, 1800).

The *Ascaris* of cats, whose cervical alae turn in abruptly at their posterior border. It has a probular posterior end. Males, 70 × 0·92 mm.; females, 101 × 1·5 mm.

P.Z.G. 2392 *Felis rufus* (American wild cat). Intestine, 3; Eastern U.S.; 9 yr. 7 mo.

25. *Toxocara canis* (Werner, 1782).

The *Ascaris* of dogs, wolves and related hosts, with cephalic alae straight and long. The tail is probular. It has short spicules, 0·91–0·99 mm. in length. Males, 50–84 × 0·75–1·17 mm.; females, 80–105 × 1·03 mm.

P.Z.G. 3800 *Canis mexicanus* (American grey wolf). Intestine, heavy infestation; born in Garden; 8 mo.
P.Z.G. —— *C. vulpes* (young red fox). Intestine; U.S.; —.
P.Z.G. 3221 (b) *C. velox* (swift fox). Intestine, 1 female; Western U.S.; 10 yr.

26. *Toxocara crenulata* (Bremser, 1824).

The *Ascaris* of wild cats, with undulating cephalic alae, and probular tail. It differs from *mystax* in the very long spicules, which are 1·95–2·05 mm. in length. Males, 22·3–54 × 0·47–0·94 mm.; females, 30–66·1 × 0·58–1·24 mm. The aoudad is a new host for this species.

P.Z.G. 5055 *Ovis tragelaphus* (aoudad). Intestine; North Africa; 3 yr. 7 mo.
P.Z.G. 4709 *Felis canadensis* (Canada lynx). Intestine, heavy infestation; northern N. America; 2 yr. 6 mo.
P.Z.G. 6463 *F. canadensis* (Canada lynx). Stomach, few only; 6 yr. 3 mo.

Genus **Toxascaris** Leiper, 1907.

27. *Toxascaris leonina* (Linstow, 1902).

An *Ascaris* having the lobules of pulp bilobed and enlarged at their extremity. Posterior extremity not probular but tapering gradually. Found nineteen times. The males are 19–55 × 0·43–0·99 mm.; the females are 45–97 × 0·52–1·23 mm. Spicules average 0·72 mm. Several lots had no numbers. Two had no host label.

P.Z.G. 3562 *Felis leo* (lion). Stomach, few only; Africa; 9 yr. 5 mo.
P.Z.G. 3938 *F. leo* (lion). Stomach, few only; Africa; 7 yr. 6 mo.
P.Z.G. 4963 *F. leo* (lion). Intestine, few only; Africa; 2 yr. 10 mo.
P.Z.G. —— 2/1/16 *F. leo* (lion). Vomited; Africa; —.
P.Z.G. —— 2/24/16 *F. leo* (lion). Vomited; Africa; —.
P.Z.G. —— 4/11/17 *F. leo* (lion). Vomited; Africa; —.
P.Z.G. 2908 *F. viverrina* (fishing cat). Stomach and intestine, about 100; India; 4 yr.
P.Z.G. 6477 *F. rufus* (wild cat). Intestine, few; Eastern U.S.; 2 yr. 8 mo.
P.Z.G. —— 1/14/16 *F. rufus* (wild cat). Vomited; Eastern U.S.; —.
P.Z.G. 3293 (b) *F. rufus texensis* (spotted wild cat). Intestine, few; Texas to California; 2 mo.
P.Z.G. —— 9/8/15 *F. rufus texensis* (spotted wild cat). Intestine, few; Texas to California; —.
P.Z.G. 6975 *F. concolor* (puma). Intestine, heavy infestation; N. and S. America; 2 yr.
P.Z.G. 7067 *F. concolor* (puma). Intestine, heavy infestation; N. and S. America; 8 yr.
P.Z.G. —— *F. concolor* (puma). Intestine; N. and S. America; —.
P.Z.G. 4775 *Canis dingo* (dingo dog). Intestine; born in Garden; 5 mo.
P.Z.G. 4867 *C. dingo* (dingo dog). Intestine, heavy infestation; born in Garden; 7 mo.
P.Z.G. 4945 *C. dingo* (dingo dog). Intestine, born in Garden; 9 mo.
P.Z.G. —— (2) Carnivora (?) —— —— —— ——.

28. *Toxascaris transfuga* (Rudolphi, 1819).

The *Ascaris* commonly found in bears. There are sixty-two pairs of papillae discernible in irregular rows on the ventral caudal surface of the male. There is a double papilla at the cloacal rim. The short spike-like spicules are 0·8 mm. in length. Males, 81·4–142 × 1·9–2·95 mm.; females, 108–166·4 × 2–3·25 mm.

P.Z.G. 8464 *Ursus maritimus* (polar bear). Small intestine, heavy infestation; Arctic regions; 19 yr. 8 mo.
P.Z.G. —— *U. maritimus* (young polar bear). Passed; Arctic regions; —.
P.Z.G. 6885 *U. syriacus* (Syrian bear). Jujenum, heavy infestation; Asia Minor; 2 yr 3. mo.
P.Z.G. 6076 *U. beringiana* (Kamchatkan bear). Intestine; Siberia; 7 yr. 6 mo.
P.Z.G. 6493 *U. beringiana* (Kamchatkan bear). Intestine, few; Siberia; 8 yr. 2 mo.
P.Z.G. 4852 *U. pruinosus* (Thibetan bear). Intestine; Eastern Tibet; 3 yr. 6 mo.
P.Z.G. —— *U. malayanus* (sun bear). Passed; Malay Peninsula, Sumatra, Borneo; —.

Genus **Parascaris** Yorke and Maplestone, 1926.

29. *Parascaris equorum* (Goeze, 1782).

Baylis and Daubney (1926) do not recognise the existence of a genus *Parascaris*. It is, however, a distinct genus on the lip characters alone. There is no difference whatever between those from the zebra and those from the Mongolian wild horse. All have the quadrangular deep-fissured lips with interlabia present. Skrjabin's (1916) species *P. zebrae* is untenable and should fall into synonymy under *P. equorum*. The males are 87–110·5 × 2·75–3·5 mm., the females 157·5–183 × 5·8–6·2 mm.

P.Z.G. —— *Equus przewalskii* (young Mongolian wild horse). Passed; Central Asia and Desert of Gobi; —.
P.Z.G. 6095 *E. burchelli* (Burchell's zebra). Intestine, heavy infestation; S. Africa; 6 yr. 1 mo.

Genus **Ophidascaris** Baylis, 1921.

30. *Ophidascaris filaria* (Dujardin, 1845).

An *Ascaris* of snakes with lips grooved at the base. Prong-like pulp in the lips. Interlabia and dentigerous ridges are present. There are forty-five pairs of papillae anterior to the cloaca. The spicules are long and equal, with a groove throughout. They measure 6·6 mm. The males measure 52–80 × ·55–1·05 mm., the females 68–105 × 0·91–2·03 mm.

P.Z.G. 3029 *Python molurus* (Indian python). Stomach and intestine, very heavy infestation; India; 6 yr. 4 mo.
P.Z.G. —— *P. molurus* (Indian python). Passed; —.
P.Z.G. 7331 *P. spilotes* (diamond snake). Intestine, very heavy infestation; Australia and New Guinea; 3 mo.
P.Z.G. 7317 *P. spilotes variegata* (carpet snake). Stomach and intestine, very heavy infestation; Australia and New Guinea; 3 mo.

Genus **Polydelphis** Dujardin, 1845.

31. *Polydelphis oculata* (Linstow, 1899).

An *Ascaris* with lips long and hexagonal. They have no interlabia, but the pulp has a pair of bipartite anterior processes present. The equal spicules

measure 5·72 mm. Ova 60 × 55 micra and thick shelled. Males, 55 × 1·5 mm.; females, 62 × 1·75 mm.

P.Z.G. 3719 *Python reticulatus* (reticulated python). Intestine, very heavy infestation; Burma to Philippines; 8 mo.

Genus **Ascaridia** Dujardin, 1845.

32. *Ascaridia petrensa*, new species, Pl. VI, figs. 27, 28, 29, 30, 31, 32; Pl. VIII, fig. 53.

Closely affiliated with *A. compar* (Schrank, 1790) and *A. cordata* (Linstow, 1906). Like the former it has nine pairs of papillae and the same host. Like the latter it has a cordate tail, a circular pre-anal sucker, and sub-equal spicules. The five or six pairs of post-anal caudal papillae are asymmetrical. The vulva is slightly anterior to the middle of the body in *cordata*, giving a ratio of 16 : 17, in *petrensa* it is at the beginning of the posterior fourth, giving a ratio of 1 : 4·3.

The mouth has three semicircular equal lips, each with a fissure on the inner surface making them lobed. The dorsal lip has two, the paired lips each one papilla. The nerve ring around the oesophagus is 0·6 mm. from the head end. These worms are of the same thickness throughout.

Male. 21·5 mm. long by 0·75 mm. wide. The club-shaped oesophagus is 2·41 mm. in length, or one-ninth of the total body length. The caudal end is cordate. The alae are supported by five pairs of lateral papillae. The chitinous sucker is circular, granular, and has a small papilla on the posterior rim. It is 225 micra in diameter. There are nine or ten pairs of caudal papillae: four pairs are pre-anal, five or six post-anal. Of the former, two pairs surround the sucker; two pairs lie just anterior to the cloaca; of these one pair is lateral. Two pairs only of the remaining papillae are sessile, the first post-anal and the next to the last. The remaining three or four pairs are lateral and asymmetrical as illustrated (Pl. VI, figs. 31, 32). Two variants were found in this species: one, with nine, another, with ten pairs of caudal papillae. The lower edge of the chitinous sucker is 0·7 mm., and the triangular cloacal orifice 0·49 mm. from the end of the tail. Spicules sub-equal, similar and each 2 mm. long. No gubernaculum is present.

Female. 38 mm. long by 1·07 mm. wide. Entire body white, curved ventrad in an arc. The body is the same thickness throughout. The oesophagus is 1·92 mm. to the constriction from the anterior end, or, one-ninth of the total length. Vulva, 29·2 mm. from the anterior; anal orifice, 0·75 mm. from the posterior end. Eggs are oval and measure 83–85 micra long by 50 micra wide. Type 4708, deposited in the Zoological Laboratory, University of Pennsylvania. Paratypes in P.Z.G.

P.Z.G. 4708 *Caccabis saxatilis chukar* (chukar partridge). Small intestine, many; Asia (Turkestan); 11 mo.

P.Z.G. Partridge cage 6/9/17 *C. saxatilis chukar* (chukar partridge). Passed; Asia (Turkestan); —.

33. *Ascaridia lineata* (Schneider, 1866).

This worm is very commonly found in domestic birds. The male is 47 × 0·93 mm. and the female 59 × 1 mm. There are ten pairs of papillae and one unpaired on the posterior rim of the sucker. The cloaca is triangular. Spicules are equal and 1·54 mm. in length.

P.Z.G. 6331 *Caccabis saxatilis chukar* (chukar partridge). Small intestine, many; Asia (Turkestan); 4 days.

34. *Ascaridia numidae* (Leiper, 1908).

This species has been shuttled back and forth between two genera. Yorke and Maplestone (1926, p. 216) place it in synonymy under *Heterakis tenuicauda* Linstow, 1883, which would imply that *numidae* has an oesophageal bulb, which it has not. *Ascaridia* is the generic designation of Miss Cram (1927, p. 96).

Males measure 20·8 × 1·22 mm.; females, 30·05 × 1·3–1·6 mm. Ten pairs of papillae are on the tail of the male: five pairs ventral, five pairs lateral. Two pairs of the former surround the cloaca. The inside diameter of the chitinous sucker is 0·16 mm. The spicules are long and equal. They are 2·22–3 mm. New host and new locality records are added.

P.Z.G. 8473 *Acryllium vulturina* (vulturine guinea fowl). Small intestine, heavy infestation; East Africa; 5 mo.

P.Z.G. 5652 *Caccabis saxatilis chukar* (chukar partridge). Small intestine, few only; Asia (Turkestan); 11 days.

35. *Ascaridia stroma* (Linstow, 1899) Railliet and Henry, 1914.

A species not often seen and not adequately described. Ten pairs of caudal papillae are present according to Cram (1927, p. 99). The present material has twelve pairs. It is placed in this species because the adanal pair and the two pairs directly posterior to it have large bases. It is the only species in the genus that has *Ascaris*-like arrow-shaped head membranes. In addition to the usual three pre-anal pairs of papillae, one adanal and six post-anal, there is one asymmetrical pre-anal pair, and one pair at the base of the adanals (Pl. IV, fig. 8). Von Linstow's (1899) illustration is very diagrammatic. The sucker has a papillar interruption. Males, 30·6 × 0·99 mm.; females, 49·8 × 1·32 mm. Diameter of sucker 0·13 mm. Spicules equal and 0·96 mm. long. Female anal orifice 0·84 mm. from the tail end. Has been reported only from the Berlin Zoological Garden and from India.

P.Z.G. 4736 *Grus* (*Tetrapteryx*) *paradisea* (Stanley crane). Intestine, many present; S. Africa; 18 days.

Family HETEROCHEILIDAE Railliet and Henry, 1915.

Genus **Porrocaecum** Railliet and Henry, 1912.

36. *Porrocaecum angusticolle* (Molin, 1860).

A bird-infesting *Ascaris* with anterior intestinal caecum 3·22 mm. long and an oblong ventriculus 0·4 mm. long. This species has the dorsal lip pulp two-

lobed and joined by a saddle. Males, 40 × 0·86 mm.; females, 99 × 1·14 mm. The anal orifice of the female is 0·5–0·6 mm. from the end of the bluntly conical tail. Fully formed embryos in punctate shell, 75 × 105 micra, can be seen in the vagina.

P.Z.G. 5641 *Buteo lineatus* (red-shouldered hawk). Intestine, heavy infestation; Eastern N. America; 3 mo.

37. *Porrocaecum depressum* (Zeder, 1800).

An avian *Ascaris* that has the lip pulp projecting in two bipartite processes and a large rounded projecting median lobe as high as the inner process, that differentiates it from *angusticolle*. Males, 67 × 0·69 mm.; females, 70 × 0·88 mm. Intestinal caecum 3·56 mm. in the male. Oblong ventriculus 0·35–0·42 mm. The anal orifice of the female is 0·13 mm. from the posterior end. Spicules in the male are equal and 1·06 mm. in length.

P.Z.G. 6992 *Buteo borealis* (red-tailed hawk). Duodenum, heavy infestation; Eastern N. America; 2 mo.

P.Z.G. 4297 *Aquila audax* (wedge-tailed eagle). Duodenum, heavy infestation; Australia; 5 mo.

Family SPIRURIDAE Oerley, 1885.

Genus **Habronema** Diesing, 1861.

38. *Habronema incerta* (Smith, 1908).

This parasite, first named from material from this garden, is still common here. Three new hosts, blue-headed pigeon, banded toucan, and the rhea, have been found. Yorke and Maplestone (1926, p. 297) erroneously spell the species name as incert*um*. Males are 7–14 mm. in length by 0·29–0·52 mm. in width; females, 9–19 by 0·42–0·59 mm. The right, bluntly rounded spicule is 0·66 mm. The left, slender, sharp one is 2·03 mm. Some of the caudal papillae are in a small group at the end of the tail. One is on the cloacal rim, and six pedunculated pairs support the caudal alae. In seven different hosts:

P.Z.G. 8618 (a) *Rhea americana* (S. American rhea). Mucosa of gizzard, many; Bolivia to Argentine; —.

P.Z.G. 1185 *Starnoenas cyanocephalus* (blue-headed pigeon). Proventricle; Florida and Cuba; 3 mo.

P.Z.G. 1610 *Platycercus eximius* (roselle parrakeet). Proventricle; Australia; 2 mo.

P.Z.G. 1346 *P. eximius* (roselle parrakeet). Proventricle; Australia; 3 mo.

P.Z.G. 6040 *Eclectus roratus* (grand eclectus). Proventricle, many present; Moluccas; 28 mo.

P.Z.G. 1290 *Chrysotis levaillanti* (Levaillant's amazon). Proventricle; Mexico to Honduras; —.

P.Z.G. 1351 *Conurus ruginosus* (brown-throated conure). Proventricle; S. America; 3 mo.

P.Z.G. 6042 *Pteroglossus torquatus* (banded toucan). Proventricle, many present; Central America; 9 mo.

Genus **Cyrnea** Seurat, 1914.

39. *Cyrnea colini* Cram, 1927.

The genus *Cyrnea* had not been reported from birds in North America until Miss Cram described her new species (p. 169) from a Georgia quail. Miss Cram sent a male and two females for comparison. Later the original

type slide was put at the writer's disposal. She overlooked two papillae, ventral, sessile, and just anterior to the cloaca. Hence, her figure of the tail of the male type is inaccurate. The males have ten pairs of papillae; nine of them support the semi-lunar transversely striated alae (Pl. IV, fig. 6). The males are 8·5 × 0·34 mm.; females, 14·5 × 0·39 mm. The divided oesophagus is long and of the same diameter throughout. The spicules are unlike and unequal. The larger, left, is thin and 1·82 mm. long; the shorter, right, is half that. The transition from the first group of five large papillae to the last group of four is well marked.

P.Z.G. 3481 (b) *Colinus virginianus texanus* (Texas bobwhite quail). Proventricle, few only; Texas; 18 days.

Family ACUARIIDAE Seurat, 1913.

Genus **Acuaria** Bremser, 1811.

40. *Acuaria* (*Dispharynx*) *resticula* new species, Pl. VII, figs. 37, 38, 39, 40, 41; Pl. VIII, fig. 46.

A new species of the sub-genus *Dispharynx* that Railliet, Henry, and Sissoff (1912) erected and to which Cram (1927) gives generic rank. She gives a generic diagnosis of *Acuaria sensu stricto* (p. 216): "6 to 8 pairs of postanal papillae." There is need of revision as she includes *A. depressa* (p. 222) with "5 pairs of these (are) postanal."

A. (*D.*) *resticula* is closely related to *A.* (*Dispharynx*) *spiralis* (Molin, 1858) and *A.* (*Dispharynx*) *laplantei* (Seurat, 1919 *a*). Because the cephalic cuticular cordons are strongly flexed, they are recurrent for a short distance only, and do not join each other. The position of the anus, the shape of the ovijector, the non-salient vulva, and the attenuated body are similar. *Resticula* differs from the other two species named as follows: the four markedly flexed cordons extend beyond the termination of the muscular portion of the oesophagus (in them only half the distance). It has a small post-cervical single papilla. (In them bicuspid and tricuspid respectively.) In addition, the spicule length and position of the vulva differ.

There are two lateral papilliform lips with two small papillae at the base of each.

Male. The males are 9 mm. long by 0·46 mm. wide. The long straight oesophagus is two-parted. An anterior muscular part, 0·9 mm. in length, joins a wider, longer, glandular part 2·5 mm. in length. Its total length is 3·4 mm. This is greater than a third of the length of the body. The cordons are 1·03–1·31 mm. in length. They recurve anteriorly at a point 0·11 mm. caudad of the oesophagus, for a distance of 0·17–0·18 mm. The tail end of the body is considerably coiled. The dissimilar and greatly unequal spicules are 0·76 and 0·22 mm. long. The position of the cloaca is 0·51 mm. from the tail end, or one-seventeenth of the body length. Nine pairs of caudal papillae support the narrow alae; five pairs of these are post-anal and four pairs are pre-anal. The post-anal papillae are in two groups of two pairs each and one

pair at the tip, as in *A.* (*Dispharynx*) *nasuta* (Rudolphi, 1819), rather than as in *spiralis* or *laplantei* in this respect.

Female. 10 mm. in length and 0·57 mm. in width. The oesophagus is 4·3 mm. long of which the muscular portion is 0·95 mm. and the glandular portion 3·35 mm. This oesophagus is nearly half the length of the body. The cordons are 1·28 mm. in length. They recurve anteriorly a short distance of 0·2 mm. from a point just caudad of the muscular portion of the oesophagus. The anus is 0·13 mm. from the blunt caudal end. The non-salient vulva is difficult to find. It is 7·3 mm. from the anterior end. It leads into a V-shaped ovijector having a large unicellular gland at its apex. The anterior vestibule of this ovijector is 0·1 mm., the posterior vestibule 0·25 mm. long. This posterior vestibule joins two uteri that loop around the intestine and which are filled with numerous ova. The ova are embryonated when deposited and measure 40–42 × 23–25 micra. Type deposited in the Zoological Laboratory, University of Pennsylvania. Paratypes in P.Z.G.

P.Z.G. 3818 *Crotophaga sulcirostris* (groove-billed ani). Proventricle, distended with heavy infestation; S.W. U.S. to N.W. S. America; 4 mo.

Family PHYSALOPTERIDAE Leiper, 1908.

Genus **Physaloptera** Rudolphi, 1819.

41. *Physaloptera caucasica* Linstow, 1902.

Two females belonging to this species were found with many *Subulura* and *Trichuris* in a baboon. These are identical with *P. mordens* Leiper, 1908. Ortlepp (1926, p. 199) has shown *mordens* is synonymous to *caucasica*. The species has a tripartite median tooth and just lateral of it an unpaired tooth. The ventral and dorsal edges of the two lateral lips have small teeth prominences just above the cephalic collarette on the inner face of the lip. There are four uteri, and the anus is terminal. The specimens are 37 and 48 mm. in length and 2 mm. wide. The vulva of the smaller specimen is 9 mm. from the anterior end. The cylindrical oesophagus is divided into a short anterior muscular part, 0·7 mm. long, and a long posterior glandular part 8·44 mm. long.

P.Z.G. 5741 (a) *Papio hamadryas* (Arabian baboon). Caecum, 2 females; Arabia and N.E. Africa; 26 mo.

42. *Physaloptera turgida* (Rudolphi, 1819).

The common stomach worm in the opossum. It has two pairs of sessile caudal papillae aligned near the tip of the tail caudad of the four costiform pairs supporting the alae. The males are 18·2–42·5 mm. in length by 0·99–1·77 mm. in width, the females 23–66·5 mm. in length by 1·04–2·67 mm. in width. The maximum sizes given are the largest on record for the species. The spicules are short, equal, and 0·47–0·6 mm. long. The cloaca is 1·33–1·53 mm. from the tail end. The anal aperture of the female is 0·31–1·19 mm. from the tail end.

P.Z.G. 4264 *Didelphys virginiana* (common opossum). Colon; Eastern and Central U.S.; 4 mo.

P.Z.G. 5091 *D. virginiana* (common opossum). Stomach, 6 present; Eastern and Central U.S.; 10 days.

P.Z.G. 5623 *D. virginiana* (common opossum). Stomach, 6 present; Eastern and Central U.S.; 11 days.

P.Z.G. 2872 *Taxidea taxus* (American badger). Stomach, 4 present; Western N. America; 11 mo.

P.Z.G. 2873 *T. taxus* (American badger). Stomach, heavy infestation; 14 mo.

P.Z.G. 8605 *Theropithecus gelada* (gelada baboon). Stomach, very heavy infestation; S. Abyssinia; 32 mo.

43. *Physaloptera multiuteri*, new species, Pl. V, figs. 15, 16, 17, 18, 19, 20, 21.

This new species of the Polydelphid group is related to *P. dilatata* Rud. 1819, on the one hand, and *P. torresi* (Travassos, 1920), on the other. It agrees with *dilatata* in the form of the lips, attenuation of the anterior end, anatomy of the female genitalia, position of the vulva, relative length of the oesophagus, and size of the ova. It is, however, readily distinguished from that species by the number of uteri, arrangement of the caudal papillae, truncation of the outer tooth, and the length and shape of the spicules. It agrees with *torresi* in the arrangement of the female genitalia, position of the vulva, and in the number and arrangement of caudal papillae. It differs from *torresi*, however, in the number of uteri, shape of the caudal alae, teeth, spicules, ova, and size.

The following table gives Ortlepp's list of characters for specific determination in order of relative importance:

Table IV.

	P. dilatata	*P. torresi*	*P. multiuteri* n.sp.
1. No. of uteri and mode of origin	12 in one, 15 in another, from outer edge of egg chamber	9–11, egg chamber directly	13 in one, 14 in another, from outer edge of egg chamber
2. No. and arrangement of male caudal papillae	10 prs., 1 unprd.; prs. 1 and 2 postanal in a row, prs. 2, 3 and 4 equally distant from each other, prs. 4 and 5 closer together than others	10 prs., 1 unprd.; prs. 1 and 2 postanal in a row, prs. 3, 4 and 5 equally distant from each other in anterior half of the tail	10 prs., 1 unprd.; prs. 1 and 2 postanal not in a row, prs. 3, 4 and 5 equally distant from each other in anterior half of tail
3. Relative length and shape of spicules	Short, equal, 655μ	Short, subequal, 430μ to 520μ	Short, unequal, right 410μ, left 670μ
4. No., shape and size of labial teeth	Outer: larger, conical, same height. Inner: semi-membranous, tripartite	Three teeth, median larger, more internal	Outer: markedly truncate. Inner: lower, semi-membranous, tripartite
5. Cuticular markings of male bursa	Longitudinal	——	Longitudinal
6. Position of vulva	In front of middle of oesophagus	Midway of oesophagus	In front of middle of oesophagus
7. Presence or absence of a cuticular sheath at posterior extremity	Absent	Absent	Absent
8. Size of eggs	39 × 27μ	42–49 × 28μ	40 × 22–24μ embryonated
9. Length of oesophagus compared to body length	♂♂: 1/4·3–1/4·8; ♀♀:1/7·5–1/8·6	♂: 1/5·4; ♀: 1/8·5	♂: 1/5·7; ♀: 1/10·7

The two large sub-triangular lateral lips bear an outer truncate tooth and an inner, smaller, semi-membranous tripartite one. There are two dome-like papillae on each lip. The attenuated head end has a cuticular collarette and the cervical surface bears loose folds of cuticle doubled-up irregularly. The oesophagus is divided into a short, anterior, muscular part, and a long, posterior, glandular part. The nerve ring surrounds the muscular part. The cervical papillae are 0·98–1·2 mm. from the anterior end. The excretory pore is 0·2 mm. posterior of the cervical papilla.

Male. 25–30 mm. long by 0·99 mm. wide. Oesophagus 4·3 mm. long or 1–5·7 mm. in the body length. The caudal alae are not sharply defined. The caudal cuticle is inflated. There are ten pairs of papillae and one pre-cloacal unpaired. Four short, costiform pairs support the alae and are equally spaced around the cloaca. Three sessile papillae are in a line immediately anterior to the cloaca of which the middle unpaired one is the largest. Two sessile pairs are immediately caudad of the cloaca. Three sessile pairs are evenly spaced farther back, the last pair being just beyond half the distance from the cloaca to the end of the tail. The cloacal aperture is 1·52 mm. from the tail end. It is in the midst of longitudinal ridges on the ventral caudal surface. The short spicules are unequal and bluntly pointed. The right is 0·41 mm. in length, the left, thinner and 0·67 mm. in length. Their origins are widely separated.

Female. 24–57 mm. in length by 0·85–2 mm. in width. The oesophagus in the larger individuals is 5·3 mm. long or 1 : 10·7 in the body length. The anterior muscular part of the oesophagus is 0·5 mm. in length by 0·15 mm. in width, and the posterior glandular part 4·8 mm. by 0·2–0·6 mm. There are thirteen uteri branching from the outer edge of the club-shaped egg chamber in a 24 mm. worm and fourteen uteri in a 57 mm. worm. Mature ova, 40 × 22–24 micra, in the vagina contain fully developed embryos. The vulva is flush with the cuticle and opens in front of the mid-oesophageal region 2·4–3 mm. from the anterior end. The sub-terminal anal orifice is 0·78 mm. from the bluntly rounded tail end.

Type deposited in the Zoological Laboratory, University of Pennsylvania. Paratypes in P.Z.G. This makes ninety-four known species in the Genus *Physaloptera*.

P.Z.G. 6403 *Ateles ater* (black spider monkey). Very many in oesophagus, a few in the stomach; Panama to Peru; 2 yr. 1 mo.

Family FILARIIDAE (Cobbold, 1864) Claus, 1885.

Genus **Dirofilaria** Railliet and Henry, 1911.

44. *Dirofilaria spinosa*, new species, Pl. V, figs. 10, 11, 12, 13, 14; Pl. VIII, fig. 47.

This species was found in association with numerous *Dipetalonema diacantha* females. It is related to *D. subcutanea* (Linstow, 1899) and *D. repens* Railliet and Henry, 1911. The size, shape and asymmetrical arrangement of the caudal papillae in *spinosa* is different from either of the above two species. The

membranous left spicule, the distance of the anus from the posterior end, the position of the vulva, and the size in general are other differences. The oesophagus is divided into an anterior muscular and a posterior glandular portion. Boulenger (1920) states that the oesophagus in the above-mentioned *D. subcutanea* is not divided. He gives the following characters for that species:

Table V.

Host... ...	*D. repens* R. and H. 1911 Dog	*D. spinosa* n.sp. Porcupine	*D. subcutanea* (Linstow, 1899) Boulenger, 1920 Porcupine	*D. subcutanea* (Linstow, 1899) Porcupine
Male:				
Dimensions: length	48–70 mm.	55–65 mm.	48–56 mm.	42 mm.
breadth	0·35–0·45 mm.	0·308 mm.	0·42 mm.	0·39 mm.
Distance from cloaca to post. end	0·075 (66–85)	0·075 mm.	0·07 mm.	—
Form of large caudal papillae	Oblong	Spherical	Elongated	—
No. and arrangement of large papillae	Asymmetrical; 2–4 on one side (pre-anal), 5 or 6 on the other of which 1 ad-anal or slightly post-anal	Asymmetrical; 4 on right side (pre-anal), 9 on left side of which 1 ad-anal and 1 post-anal	Symmetrical; 4 prs. pre-anal, 1 pr. post-anal, 4 smaller prs. post-anal	Symmetrical; 6–7 prs. pre-anal, 6 prs. post-anal
Spicule length:				
larger	0·465–0·59 mm.	0·3 mm.	0·38–0·42 mm.	0·44 mm.
smaller	0·185–0·206 mm.	0·105 mm.	0·13–0·14 mm.	0·11 mm.
Ratio larger to smaller spicule	2·5 : 1	3 : 1	3 : 1	4 : 1
Female:				
Dimensions: length	100–170 mm.	124–166 mm.	160–175 mm.	195 mm.
breadth	0·46–0·65 mm.	0·572–0·59 mm.	0·7 mm.	0·57 mm.
Distance of vulva from anterior end	1·15–1·6 mm.	2·17 mm.	1·19–2·4 mm.	1·23 mm.
Distance of anus from posterior end	0·05–0·09 mm.	0·2 mm.	0·35 mm.	—
Size of embryo or ova	Emb. 0·3–0·36 × 0·006 mm.	Ova 0·023 × 0·015 mm.	Ova 0·022 × 0·015 mm.	Ova 0·018 × 0·014 mm.

It is evident that Boulenger was misled by Hall (1916, p. 182), and, in fact, he described and illustrated (Pl. VIII, figs. 48, 49) a species that should have been designated as new. He stated (p. 493): "My account will be found to differ in some respects from that given by von Linstow...." The papillae are arranged differently. The ratio of the larger spicule to the smaller (Table V), and the position of the vulva are very different. These characters are of specific value. Accordingly the writer designates *D. subcutanea* (Linstow, 1899) Boulenger, 1920, as *Dirofilaria hystrix* (new name).—From the subcutaneous tissue of a Canadian porcupine *Erethizon dorsatus*. The specific name under which Boulenger put his specimen is already a doubtful homonym of *F. subcutanea* Parona, 1894. A discussion of their status can be found in Hall (1916, pp. 182–3).

These filamentous white threadworms have no lips. They have six papillae around the simple mouth opening: two lateral, and four sub-median. The vulva is post-oesophageal. The oesophagus has two parts. The nerve ring constricts the muscular oesophagus midway, 0·32 mm. from the anterior. The lateral cervical papillae are located at the junction of the muscular and glandular parts of the oesophagus.

Male. 55–66 mm. long by 0·3 mm. wide. The oesophagus is 1·05 mm. long, hence, one-fifty-second of the body-length. The anterior muscular part of this oesophagus is 0·4 mm. long. The pedunculated asymmetrical caudal papillae have large orange-shaped bases. There are nine in a row on the left ventral surface. The most anterior one is the largest and is 52 micra in diameter. They become smaller caudad, the last being but 15 micra in diameter. There are four in a row on the right ventral surface, the most anterior the largest, then two medium-sized and equal in diameter and, finally, one small one. There are four sessile pairs and one unpaired between the cloaca and the caudal end of the body. They form two groups, the larger in a row across the tail. The spicules are unequal in length but of the same width. The short spike-like one on the right is 0·1 mm. long, left one three times as long. The latter has a tubular base, and from more than a third of the way caudad to its posterior end it is membranous. It tapers to a fine point. The slit-like cloacal aperture is 0·07 mm. from the tip of the tail. The tail is curled corkscrew fashion.

Female. 124–166 mm. long by 0·57–0·59 mm. wide. The short oesophagus is 1·37 mm. in length. The salient vulva is post-oesophageal and 2·17 mm. from the anterior end. This distance from the anterior end gives a ratio to the maximum body length of 1 : 76. The anal pore is 0·2 mm. from the tail end. The tail is bluntly rounded and curved dorsad. The tip has a single medium-sized papilla on it. The ova measure 23 × 15 micra. Reported from the intestine. Filarids are commonly found in the connective tissue and body cavities. Type 6190. Deposited in the Zoological Laboratory, University of Pennsylvania. Paratypes in P.Z.G.

P.Z.G. 6190 *Erethizon dorsatus dorsatus* (Canadian porcupine). Intestine, heavy infestation Eastern N. America; 20 mo.

P.Z.G. 5371 *E. dorsatus dorsatus* (Canadian porcupine). Peritoneum; 9 mo.

Genus **Saurositus** Macfie, 1924.

45. *Saurositus agamae* Macfie, 1924.

Taken from a cyst in the coelomic cavity of a snake. A slender worm that was first found in a lizard *Agama colonorum*. It is the only species placed in the genus. It has two lateral and four sub-median head papillae. The tail of the male is coiled into a spiral. The vulva is just posterior to the oesophagus. The sub-equal spicules are 0·11 and 0·13 mm. long. Males, 45 × 0·2 mm.; females, 90·9 × 0·35 mm. This is a new host and locality.

P.Z.G. —— *Ancistrodon contortrix* (copperhead snake). Coelomic cavity; Eastern and Southern U.S.; —.

Genus **Setaria** Viborg, 1795.

46. *Setaria labiato-papillosa* (Alessandrini, 1838).

Females only. The head of this common form has two lateral and four pairs of sub-median papillae. It is surrounded by a chitinous peribuccal ring that is prolonged anteriorly to form notched lip-like prominences. The anterior sub-median pairs of papillae are spinous. The specimens are 96–107 mm. long by 0·82–0·85 mm. wide. A pair of lateral appendages are near the tip of the tail. The tail ends in a knob-like process composed of a group of papillae. The anal orifice is 0·72–0·74 mm. from the tail end and vulva 0·83–0·95 mm. from the anterior end. In the host list by Thwaite (1927) ? *Bison americanus* is included. The finding of *labiato-papillosa* by the writer in the American bison removes any doubt of its occurrence in that host. The yak is a new host.

P.Z.G. 5884 *Bison americanus* (American bison ("Buffalo")). Intestine, 2 only; N. America; 15 yr. 4 mo.

P.Z.G. 4203 *Poephagus grunniens* (yak). Intestine, 2 only; born in Garden; 34 mo.

Genus **Dipetalonema** Diesing, 1861.

47. *Dipetalonema diacantha* (Molin, 1858).

Differs from *D. gracile* in the bifid female tail end, shape of the winged spicules and in size. This is 32 × 0·17 mm. in the male, and 61 × 0·29 mm. in the female. Tail in a loose spiral. The winged spicules are unlike and unequal. They are 0·12 and 0·22 mm. in length. There are six head papillae of which the two larger laterals project forward.

P.Z.G. 3245 *Erethizon dorsatus dorsatus* (Canadian porcupine). Peritoneum; Eastern N. America; 13 mo.

P.Z.G. 6190 (a) *E. dorsatus dorsatus* (Canadian porcupine). Peritoneum, heavy infestation; 20 mo.

48. *Dipetalonema gracile* (Rudolphi, 1809).

The very common long white threadworm. The males measure 79–195 × 0·33–0·44 mm., the females 175–250 × 0·43–0·6 mm. The spicules are unequal, the short one being 0·19 mm., the long one 0·91 mm. long. The tail of the male is twisted. The vulva is at the middle of the oesophagus, 0·75–1·19 mm. from the anterior end. This is a common parasite of monkeys and has been listed from more than twenty-five species.

P.Z.G. 6448 *Cebus albifrons* (white-fronted cebus). Peritoneum, few only; Guyaquil, Upper Amazon; 22 mo.

P.Z.G. 6478 *C. fatuellus* (brown cebus). Peritoneum, few only; Brazil, Guiana; 13 mo.

P.Z.G. 5354 *C. hypoleucus* (white-throated cebus). Peritoneum, 25 present; Central America; 7 mo.

P.Z.G. 7068 *C. hypoleucus* (white-throated cebus). Peritoneum, 25 present, Central America; 1 yr.

P.Z.G. 8462 *Lagothrix lagothrix* (Humboldt's woolly monkey). Peritoneum, heavy infestation; Brazil, Columbia; 4 mo.

Genus **Dicheilonema** Diesing, 1861.

49. *Dicheilonema rheae* (Owen, 1843).

The writer follows Baylis (1926) in the generic designation. Baylis has shown *Contortospiculum* to be synonymous with *Dicheilonema*. The males are 98–179·8 × 0·8–1 mm., the females 335–446 × 1·2–2 mm. The thin caudal alae are supported by five pairs of large pedunculated papillae. In addition there is a sessile group of three pairs at the tail end. The spicules are very unequal: left, 1·26 mm. long, twisted and winged; right, much shorter, 0·4 mm., and winged. No gubernaculum. Vulva at junction of anterior and posterior oesophagus. The atrophied anus is 0·22 mm. from the tail end.

P.Z.G. 8618 (b) *Rhea americana* (S. American rhea). Mesenteric, heavy infestation; Bolivia to Argentina; —.

P.Z.G. 9015 *R. americana* (S. American rhea). Mesenteric and peritoneum, heavy intestation; 2 yr.

Genus **Diplotriaena** Railliet and Henry, 1909.

50. *Diplotriaena tricuspis* (Fedtsch. 1883).

Females only. They measure 104 × 0·85 mm. and are attenuate at the extremities. Lateral chitinous tridents, reaching back 0·1–0·14 mm. from the mouth, are present. The muscular part of the oesophagus is 0·26 mm. long and 0·06 mm. wide, and the glandular part 4·5 mm. long and 0·15 mm. wide. The vulva is 0·43 mm. and the nerve ring is 0·17 mm. from the head end. The anal pore is sub-terminal of the blunt tail end. The ova, 32 × 45 micra in size, contain fully developed embryos.

P.Z.G. 6014 *Cyanocorax chrysops* (pileated jay). Abdomen, several; Brazil, Uruguay; 3 mo.

51. *Agamofilaria* Stiles, 1907. Larva; Pl. VIII, figs. 42, 43.

Larvae of a form that becomes 300–350 mm. long when adult were found in the opossum. They are white, and measure 92 × 0·3 mm. They are finely striated and have two large lateral head papillae 0·01 mm. in height. The mouth is an oval slit 0·02 mm. wide and opens into a small vestibule. The oesophagus is long and narrow and tubular throughout. The vulva is in the oesophageal region, 0·61 mm. from the head end.

P.Z.G. 6392 *Didelphys philander* (philander opossum). Peritoneum, 21 found; North-Eastern S. America; 3 mo.

Records of the occurrence of Roundworm Parasites in the vicinity of Philadelphia, other than in the Philadelphia Zoological Garden, and including one new species follow:

Family TRICHOSTRONGYLIDAE Leiper, 1912.

Genus **Nippostrongylus** Lane, 1923.

52. *Nippostrongylus muris* (Yokogawa, 1920).

These are small red, curled, Nematodes with an asymmetrical male bursa, and a bell-shaped female tail. First reported from Baltimore, Md., this is the second record of the species. Males, 2·93 × 0·09 mm.; females, 4·33 × 0·11 mm.

C. Coll. 108 (a) *Mus norvegicus* (Norwegian wild rat). Duodenum, 291 found; Philadelphia. Collector, Prof. D. H. Wenrich.

Family DIOCTOPHYMIDAE Railliet, 1915.

Genus **Eustrongylides** Jägerskiöld, 1909.

53. *Eustrongylides wenrichi*, new species, Pl. VIII, figs. 55, 56, 57, 58.

The pre-adult stage of a new species the adult characters of which are evident within the last cuticle to be moulted. Found in stream pike, brook-trout, calico bass, sunfish, and frog. They are larger than many adults of related species reported from birds. Because there are no incisions on the margin of the bursal cup and because of the number of the head papillae *wenrichi* is related to *E. ignotus* Jägerskiöld, 1909. The latter has been found in Washington, D.C. (1926) by Chapin.

In 1924 Ciurea found similar forms in certain fish (*Barbus fluviatilis*, *Lota lota*, *Esox lucius*, and *Perca fluviatilis*) in the Danube which he regards as *Eustrongylides larvae*. He describes them (Cram, 1927, p. 369): "relatively large, 28 to 70 mm. long by 264 to 539 micra wide, rose-red or brown-red in colour. On each side of the body near the anterior end is a row of small lateral papillae. The mouth aperture has the form of a cleft and has about it 3 small pointed papillae on each side and beyond these 3 large papillae on each side. The larvae have tails of 2 types, one enlarged near the end and regarded as that of the male, and the other rounded off without enlargement and regarded as that of the female." His description and illustrations are the same as for this new species. The kind, number, and arrangement of the head papillae are the same. They are of the same colour, size, shape, and host. Because of this similarity the writer places Ciurea's larvae under this new species, described here.

Red worms, with the coarse cuticle striated heavily. No lips. Mouth opening just a cleft 45 micra long. There is a rectangular border around the mouth. There are twelve head papillae; three sharply pointed papillae are on each side of and close to the mouth and three larger, blunter papillae on each side in an outer circle. The head end is slightly attenuated. On each side of the body is a row of small, evenly spaced, lateral papillae on both male and female. An oesophagus 7 mm. long follows an oral cavity 0·89 mm. deep. This oesophagus has several lateral folds and maintains the same width throughout. The nerve ring is at the line of demarcation between the oral cavity and the oesophagus. The tail of the male bulges outward just before the truncated terminus, while that of the female does not.

Male. Males from the stream pike are 69·1 mm. long by 0·71 mm. wide. Males from the frog, identical in all other respects are 106 mm. long by 0·86 mm. wide. The muscular bursal cup of the male has the appearance of a vacuum cup. The loosely inflated cuticle of the last moult folds over the unbroken rim. The diameter of this rim is 0·28 mm. Within this, surrounding the protrusible terminal cloacal orifice, is another smaller bursal cup whose rim is 0·11 mm. in diameter. Before the bulge the end of the body measures 0·38 mm. in width. The bulge is 0·5 mm. wide. It quickly curves into the cup. There are two

symmetrical rows of lateral papillae that continue throughout the length of the body. Spicule, long and slender, enclosed in an internal sheath.

Female. Females from the stream pike measure 72·6–77·7 mm. long by 0·81–0·88 mm. wide. Females from the frog are 123 mm. long by 0·96 mm. wide. The truncated tail is without enlargement. Both anus and vulva are terminal. The inflated cuticle of the last moult overlaps both. Evenly spaced caudal papillae form a continuation of the lateral papillae.

It is conceded by Jägerskiöld that the primary hosts are fish-eating birds and that a fish is the secondary host, although such a life-cycle has not been proven. The frog in this case is but an aberrant host. This species is noteworthy because of the large size of the individuals and a new host for the genus.

The present material has been referred to a new species named in honour of Prof. D. H. Wenrich. Type No. 109, deposited in the Zoological Laboratory, University of Pennsylvania.

C. Coll. 109 *Esox americanus* (stream pike). Coelomic cavity, 5 males, 7 females; Philadelphia City Aquarium.

C. Coll. 154 *Salvelinus fontinalis* (American charr or brook-trout). Coelomic cavity; 5 females; Philadelphia City Aquarium.

C. Coll. 151 *Pomoxis sparoides* (calico bass). Coelomic cavity, 2 males, 3 females; Philadelphia City Aquarium.

C. Coll. 146 *Enneacanthus gloriosus* (nine-spined sunfish). Coelomic cavity, 6 females; Philadelphia City Aquarium. Collector, Dr Henry Winsor.

C. Coll. 122 *Rana catesbiana* (bull frog). Mesentery, peritoneum and liver encystment, 2 males, 2 females; Vivarium, University of Pennsylvania, Philadelphia. Collector, Prof. D. H. Wenrich.

Family OXYURIDAE Cobbold, 1864.

Genus **Oxyuris** Rudolphi, 1803.

54. *Oxyuris equi* (Shrank, 1788).

One female only, much macerated. The hexagonal mouth with teeth among bristles, the head papillae, the chitinised pouch, and the attenuated whip-shaped posterior of the body, place it in this species. Length, 122·19 mm. Diameter of the anterior end is 3·25 mm.; of the middle 0·98 mm. and of the tail end 0·2 mm.

C. Coll. 102 *Equus caballus* (horse). Passed; Cheyney, Pennsylvania. Collector, Prof. P. P. Calvert.

Family HETERAKIDAE Railliet and Henry, 1914.

Genus **Heterakis** Dujardin, 1845.

55. *Heterakis gallinae* (Gmelin, 1790).

Common in fowls and ducks. Males, 7·19 × 0·25 mm.; females, 8·04 × 0·36 mm.

C. Coll. 103 *Gallus bankiva* (chicken). Caeca, light infestation; Philadelphia. Collector, Dr W. W. Weir.

56. *Heterakis spumosa* Schneider, 1866.

From fourteen wild rats (*Mus norvegicus*), trapped during the winter of 1927–8 by Prof. D. H. Wenrich, in and around the Zoological Laboratory of

the University of Pennsylvania, three different nematode species were taken (see also No. 52 and No. 62). From a wild black rat (*Epimys rattus*) ova of *Trichuris muris* (Schrank, 1788) were taken. Males of *H. spumosa* are 5·76–6·81 × 0·25–0·28 mm.; females, 6·9–7·7 × 0·26–0·32 mm. The male tail with the great ventral cuticular thickenings and ten pairs of papillae make it easily recognisable.

C. Coll. 107 and 108 (b) *Mus norvegicus* (Norwegian wild rat). Duodenum and small intestine, 67 found; Philadelphia.

Family ASCARIDAE Baird, 1853.

Genus **Ascaris** Linnaeus, 1758.

57. *Ascaris lumbricoides* Linnaeus, 1758.

Males, 150·5–161·5 × 3–3·9 mm.; females, 182–205·4 × 3·45–4·1 mm.

C. Coll. 120 *Sus scrofa* (pig). Intestine 2 males, 9 females; Abattoir, Philadelphia. Collector, Preparator Mr H. Walters.

58. *Ascaris vitulorum* Goeze, 1782.

Males, 121 × 2·37 mm.; females, 141·7–147·5 × 3·5–4·16 mm. Of the five pairs of post-cloacal papillae one pair is double.

C. Coll. 104 (a) *Bos taurus* (calves). Intestines, very numerous; Abattoir, Philadelphia.

Genus **Toxocara** Stiles, 1905.

59. *Toxocara canis* (Werner, 1782).

One female only, 77·56 × 2·02 mm.; secured by use of anthelmintic S. and C. given two hours after castor oil.

C. Coll. 112 *Canis familiaris* (dog). Passed; Philadelphia. Collector, Dr Vera Koehring.

60. *Toxocara mystax* (Zeder, 1800).

Very common in cats prepared for student dissection in the Zoological Laboratory, University of Pennsylvania. Males, 63–74·5 × 0·99–1·21 mm.; females, 72·5–85·7 × 1–1·7 mm.

C. Coll. 113 *Felis domestica* (cat). Intestine; Philadelphia. Collector, Preparator Mr H. Walters.

Genus **Parascaris** Yorke and Maplestone, 1926.

61. *Parascaris equorum* (Goeze, 1782).

The synonym *Ascaris megalocephala* Cloquet, 1824, is more familiar. Males, 176–227 × 4·3–4·5 mm.; females, 176·5 × 4·75 mm.

C. Coll. 118 *Equus caballus* (horse). Intestine, 2 males, 1 female; Philadelphia. Collector, Preparator Mr H. Walters.

Family SPIRURIDAE Oerley, 1885.

Genus **Protospirura** Seurat, 1914.

62. *Protospirura muris* (Gmelin, 1790).

One female only of this species, 18 × 1·14 mm., was found in a rat caught by Prof. D. H. Wenrich as previously mentioned.

C. Coll. 108 (d) *Mus norvegicus* (Norwegian wild rat). Duodenum; Philadelphia.

VIII. SUMMARY.

The Helminths herein reported are of importance because of their economic and taxonomic relations. To point this out, it is only necessary to mention *Haemonchus*, whose relation to the Bovidae and Ovidae is equivalent to those of *Necator* and *Ancylostoma* to the human host.

Some are well-known roundworms having a world-wide distribution as parasites of animals, and are listed only on account of locality and museum interest; others, especially certain rare forms from uncommon hosts, are of greater interest and are discussed more fully. Certain details, at one time thought not to be necessary and hence neglected are added because to-day they are indispensable for specific designation.

Because of the abundance of material, it has been possible to supplement accounts given by other authors and to correct some errors due, no doubt, to poor or too few specimens. Several changes in systematic position of known forms and confirmation of others are made.

A new name (*D. histrix*) is proposed for *Dirofilaria subcutanea* (Linstow, 1899) Boulenger, 1920. *Eustrongylides larvae*, of Ciurea, J. 1924, are included under the pre-adult stage of a new species (*Eustrongylides wenrichi*) because both are alike and their characters fit no other known species. New information is added about *Trypanoxyuris trypanuris* Vevers, 1923, and *Cyrnea coloni* Cram, 1927, amending their descriptions.

Descriptions and illustrations of seven new species in as many genera are given. They are *Acuaria* (*Dispharynx*) *resticula* from a groove-billed ani, *Ascaridia petrensa* from a partridge, *Dirofilaria spinosa* from a porcupine, *Physaloptera multiuteri* from a monkey, *Subulura pennula* from a quail, *Spironoura procera* from a terrapin, and the above-mentioned pre-adult *Eustrongylides wenrichi* from a stream pike, brook-trout, calico bass, sunfish, and a frog.

In all there are 162 determinations in 38 genera and 56 species, including new ones, from 150 hosts involving 117 different host species.

Superparasitism and significant cases of parasitism are pointed out because they are of interest to one concerned with incidence and degree of infestation. Numerous records of new host-parasite relations and new localities will be of interest to students of distribution.

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EXPLANATION OF PLATES IV—VIII.

All drawings were made with the aid of a camera lucida.

PLATE IV.

Fig. 1. *Ancylostoma pluridentatum*. Dorsal view of the head of the male.

Fig. 2. *Murshidia falcifera*. Lateral view of the tail of the male.

Fig. 3. *Poteriostomum imparidentatum*. Lateral view of the tail of the male showing ventral ray separate from lateral.

Fig. 4. *Brachyclonus indicus*. Dorsal view of the male dorsal bursal ray. Tridigitate.

Fig. 5. *Monodontella giraffe*. Dorsal view of the asymmetrical male bursa.

Fig. 6. *Cyrnea colini*. Ventral view of the tail of the male showing papillae.

Fig. 7. *Cruzia tentaculata*. Lateral view of the tail of the male showing variation in caudal papillae.

Fig. 8. *Ascaridia stroma*. Ventral view of the tail of the male showing papillae arrangement and large bases.

Fig. 9. *Trypanoxyuris trypanuris*. Lateral view of the tail of the male.

PLATE V.

Fig. 10. *Dirofilaria spinosa* sp. nov. Lateral view of the head of the female.

Fig. 11. *D. spinosa* sp. nov. Lateral view of the head of the male.

Fig. 12. *D. spinosa* sp. nov. Lateral view of the vagina of the female.

Fig. 13. *D. spinosa* sp. nov. Lateral view of the tail of the female.
Fig. 14. *D. spinosa* sp. nov. Ventral view of the tail of the male.
Fig. 15. *Physaloptera multiuteri* sp. nov. Lateral view of the head of the female.
Fig. 16. *P. multiuteri* sp. nov. Ventral view of the head of the male.
Fig. 17. *P. multiuteri* sp. nov. Fourteen uteri of the female.
Fig. 18. *P. multiuteri* sp. nov. Thirteen uteri of the female.
Fig. 19. *P. multiuteri* sp. nov. Lateral view of the lips and teeth of the male.
Fig. 20. *P. multiuteri* sp. nov. Ventral view of the tail of the male.
Fig. 21. *P. multiuteri* sp. nov. Lateral view of the tail of the female.

PLATE VI.

Fig. 22. *Spironoura procera* sp. nov. Lateral view of the head of the female.
Fig. 23. *S. procera* sp. nov. *En face* view of the head of the male.
Fig. 24. *S. procera* sp. nov. Lateral view of the tail of the male.
Fig. 25. *S. procera* sp. nov. Lateral view of the tail of the female.
Fig. 26. *Subulura distans*. Ventral view of the tail of the male.
Fig. 27. *Ascaridia petrensa* sp. nov. *En face* view of the head of the male.
Fig. 28. *A. petrensa* sp. nov. Ventral view of the lips of the female.
Fig. 29. *A. petrensa* sp. nov. Ventral view of the head of the female.
Fig. 30. *A. petrensa* sp. nov. Lateral view of the tail of the female.
Fig. 31. *A. petrensa* sp. nov. Ventral view of the tail of the male.
Fig. 32. *A. petrensa* sp. nov. Ventral view of the tail of the male showing a variation from the type.

PLATE VII.

Fig. 33. *Subulura pennula* sp. nov. Ventral view of the head of the male.
Fig. 34. *S. pennula* sp. nov. Ventral view of the tail of the male.
Fig. 35. *S. pennula* sp. nov. Lateral view of the tail of the female.
Fig. 36. *S. pennula* sp. nov. *En face* view of the head of the female.
Fig. 37. *Acuaria* (*Dispharynx*) *resticula* sp. nov. Lateral view of the tail of the female.
Fig. 38. *A.* (*Dispharynx*) *resticula* sp. nov. Lateral view of the tail of the male.
Fig. 39. *A.* (*Dispharynx*) *resticula* sp. nov. Lateral view of the head of the male.
Fig. 40. *A.* (*Dispharynx*) *resticula* sp. nov. Ventral view of the lips of the female.
Fig. 41. *A.* (*Dispharynx*) *resticula* sp. nov. Ventral view of the head of the female.

PLATE VIII.

Fig. 42. *Agamofilaria* (larva). Lateral view of the head.
Fig. 43. *Agamofilaria* (larva). Ventral view of the head.
Fig. 44. *Brachyclonus indicus*. Lateral view of the head of the male showing denticles.
Fig. 45. *B. indicus*. Dorsal view of the head of the male showing denticles.
Fig. 46. *Acuaria* (*Dispharynx*) *resticula* sp. nov. Ovijector of the female.
Fig. 47. *Dirofilaria spinosa* sp. nov. *En face* view of the head of the female.
Fig. 48. *Dirofilaria subcutanea*. Ventral view of the tail of the male after von Linstow, 1899.
Fig. 49. *D. subcutanea*. Lateral view of the tail of the male after Boulenger, 1920. *pr.* pre-cloacal papillae; *po.* post-cloacal papillae; *spi.* spicules.
Fig. 50. *Trypanoxyuris trypanuris*. Ventral view of the lips of the female.
Fig. 51. *T. trypanuris*. Ventral view of the head of the female showing inflated cuticle.
Fig. 52. *Subulura pennula* sp. nov. Semi-lateral view of the head of the male.
Fig. 53. *Ascaridia petrensa* sp. nov. Ovum.
Fig. 54. *Subulura pennula* sp. nov. Ventral view of the head of the female.
Fig. 55. *Eustrongylides wenrichi* sp. nov. *En face* view of the head of the male.
Fig. 56. *E. wenrichi* sp. nov. Lateral view of the head of the female.
Fig. 57. *E. wenrichi* sp. nov. Lateral view of the tail of the female. *v.* vagina; *c.* cuticle of last moult.
Fig. 58. *E. wenrichi* sp. nov. Ventral view of the tail of the male. *s.* spicule; *c.* cuticle of last moult.

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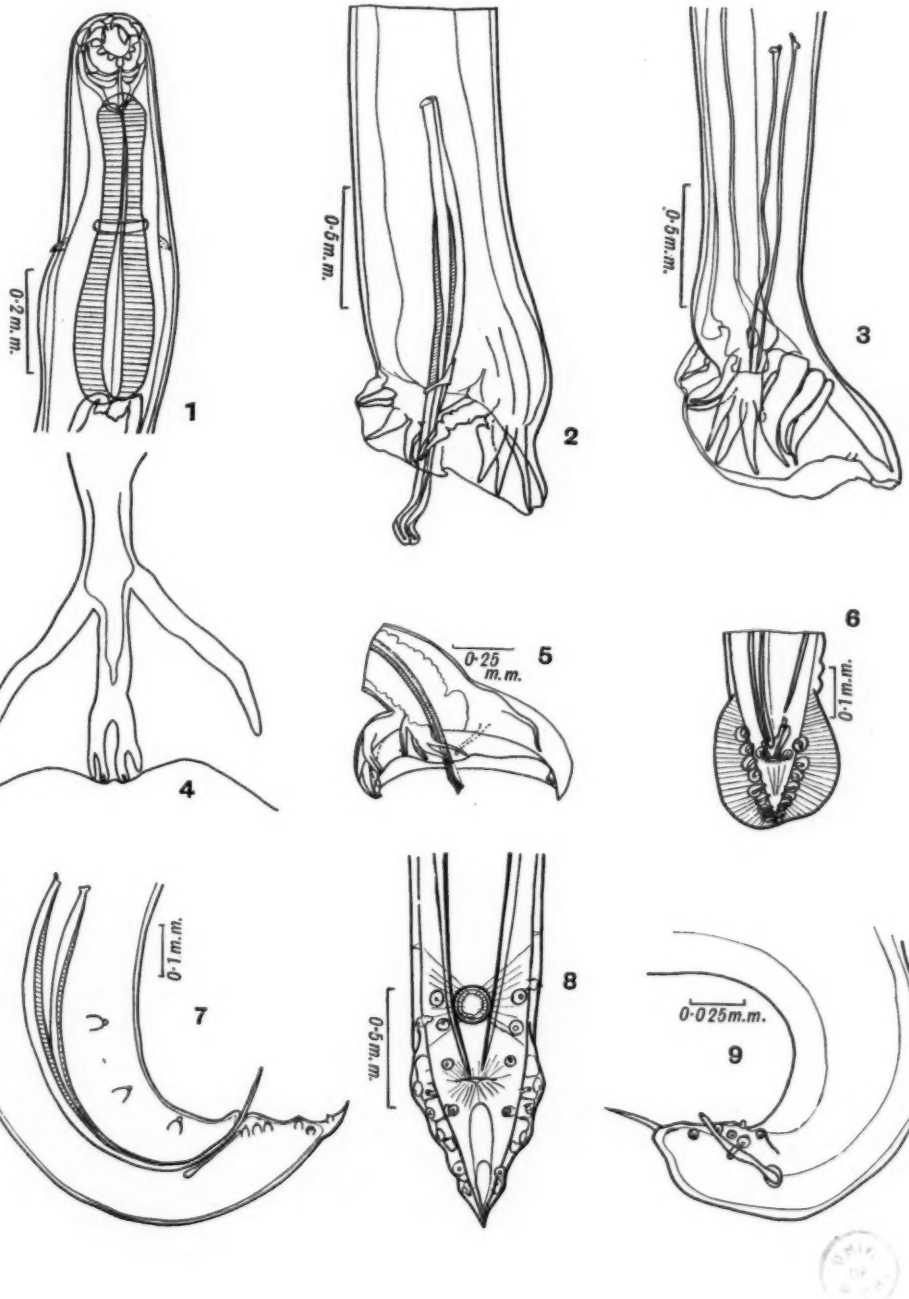
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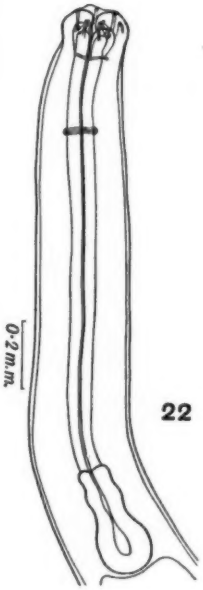

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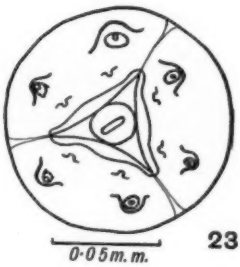

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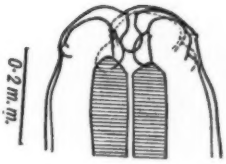

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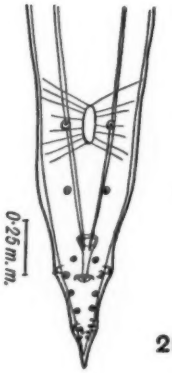

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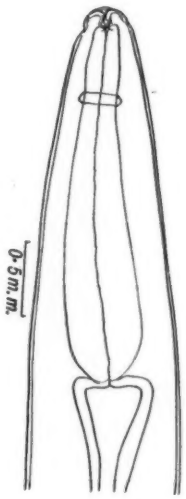

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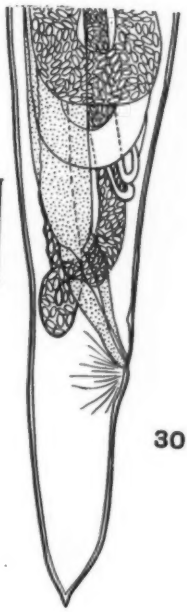

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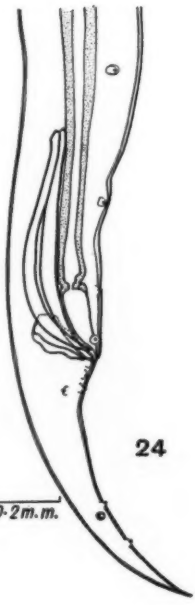

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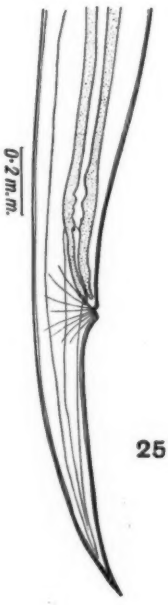

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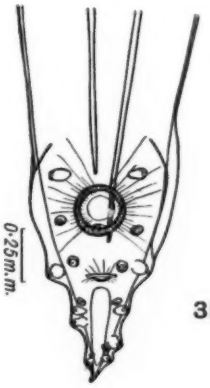

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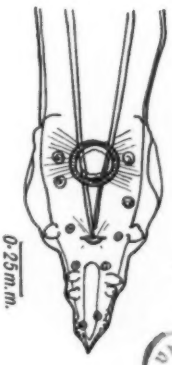

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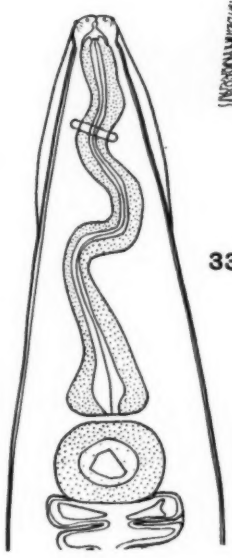

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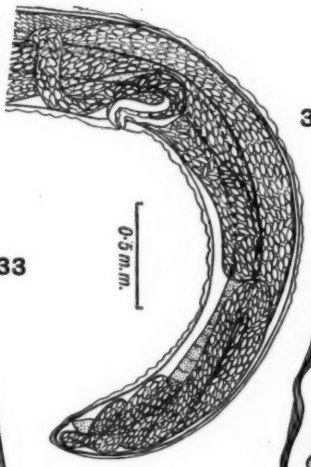

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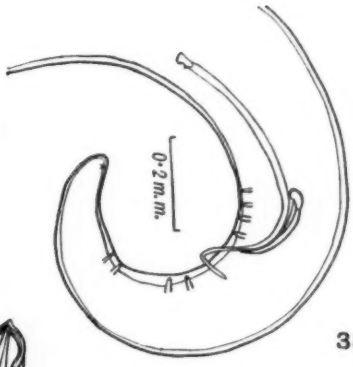

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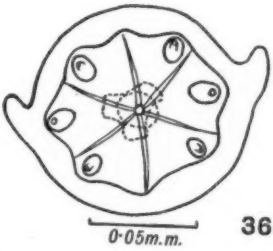

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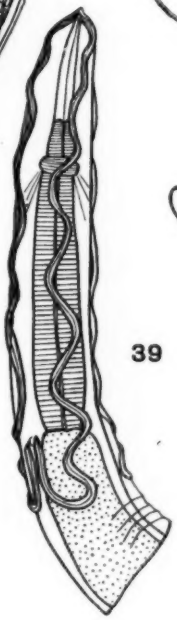

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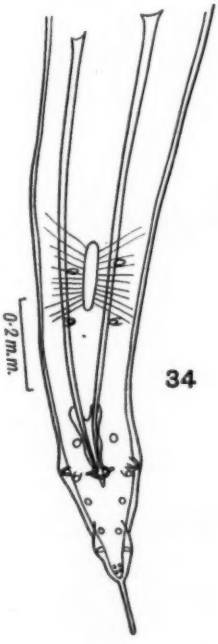

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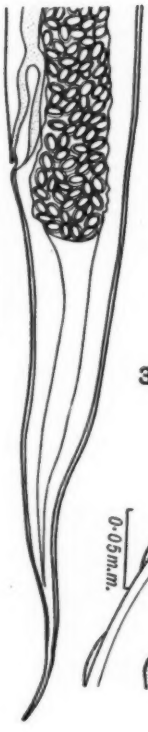

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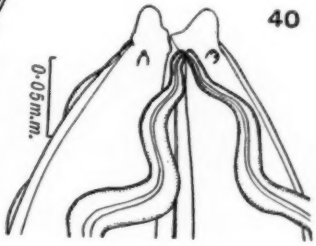

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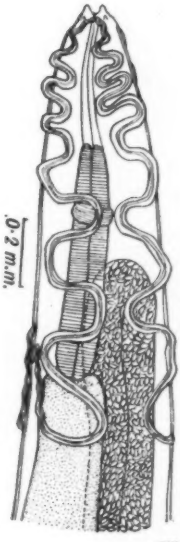

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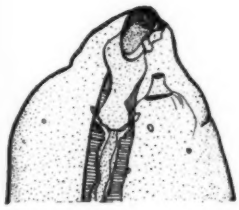

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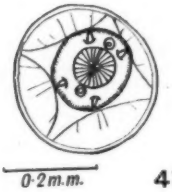

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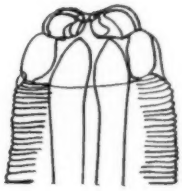

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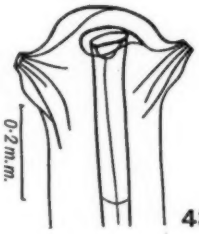

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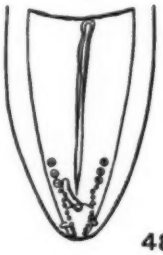

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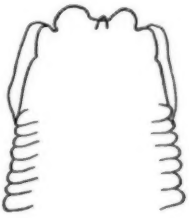

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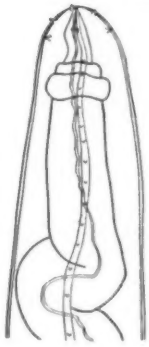

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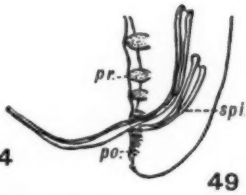

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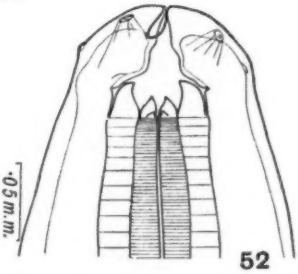

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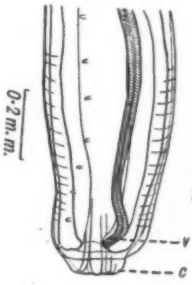

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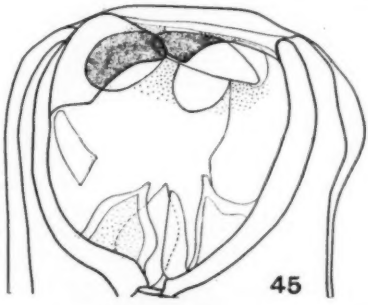

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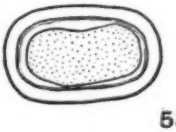

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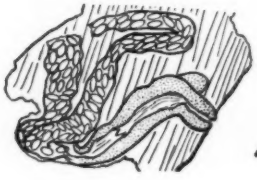

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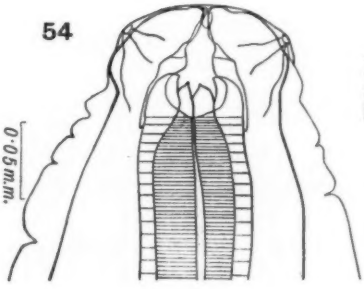

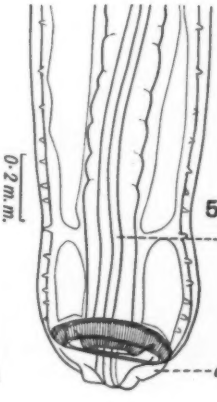

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ON A CHALCIDOID PARASITE BRED FROM A FLEA LARVA.

By JAMES WATERSTON, D.Sc.

(With 1 Text-figure.)

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For the opportunity to examine the material on which the following descriptions are based I am indebted to the courtesy of Dr P. A. Buxton and Miss E. Sikes of the London School of Tropical Medicine, where the life-history of certain fleas is being studied. A detailed examination of the parasite showed it to be referable to the Pirenine tribe of the Miscogasteridae —a family of Chalcidoid wasps. The genus and species appeared to be new. The fact, however, that the parasite had been bred from a species of flea known to have been recently introduced into Britain suggested the advisability of comparison with American representatives of the Pirenine group. This has now been done by my friend Mr A. B. Gahan of the Bureau of Entomology, Washington, D.C., to whom my hearty thanks are due. As the result we are agreed that a new genus is required. *Bairamlia* n.g. has obvious affinities with *Pirene* Hal., but differs in antennal, neurational and propodeal characters. *Ecrizotes* Forst. (*fide* A. B. Gahan) may also be a nearly related genus. As regards the species, I am satisfied that it is very different from anything described by Walker of which material is still extant in the British Museum. Mr Gahan, however, tells me that *Pirene marylandensis* Gir. (*Canad. Ent.* 48, p. 116, 1916) is a congeneric though distinct species.

Family MISCOGASTERIDAE.

Tribe Pirenini.

Bairamlia n.g.

Head. Transverse, wider than thorax, eyes rather small, vertex wide. Temples well developed, occiput strongly concave not margined; genae long; median clypeal lobe short and straightly truncate. Toruli very low down, nearly on apparent mouth edge. No genal keel or line.

Mandibles similar, 4-dentate; labrum simple, transverse; palpi normal. Antenna, scape, pedicel, 3 ring joints, 5 in funicle and 3 in club, funicle and club widening very little.

Thorax. Pronotum campanulate, transversely finely margined. Parapsidal furrows complete. Scutellum frenate. Propodeon truncate triangular with

broad nucha, considerably extending beyond the insertion of hind coxa. Fine median keel, no lateral plicae, spiracles small, oval. Wings; submarginal greater than marginal greater than post-marginal greater than radius.

Abdomen oval acuminate, shortly petiolate; the petiole nearly perpendicular. First tergite basally shortly crenulate.

Genotype the following species:

Bairamlia fuscipes n.sp.

A blackish shining species (♂, ♀) with sub-metallic reflections at most on discs of thorax and abdomen. *Antennae* and *femora* concolorous with body. *Tibiae* piceous; in mid and fore legs paler at base and apex, in hind legs only narrowly at base.

Fifth tarsal joint piceous, the others not quite so dark, and paler at the articulations. Wings hyaline, veins pale brown. [In some individuals there is a faintly tinted transverse streak connecting the hairs standing in the position of the vanished basal vein. See fig. 1, *a*.]

Head. *From in front* broadly oval, wider (8 : 7) than deep. Eyes occupying one-half of the depth; orbits sub-parallel, very slightly divergent ventrally, separated by about two-thirds the breadth. Genae strongly convergent towards mouth edge. Genal space long, five-sevenths of the depth of the eye. Distance between anterior ocellus and toruli equal to that between the eyes. The toruli, small sub-circular, one and two-thirds of the diameter apart, are set practically on the apparent mouth edge, the clypeus being inturned and partly horizontal. The clypeal and inter-torular swellings are separated by a shallow furrow; eyes bare.

From above more than one-quarter wider than thorax, and measured across eye, about two and a half (17 : 7) times as broad as long, but in middle shorter (17 : 8) as the occiput is strongly concave. Temples posteriorly convergent.

Surface generally smooth and shining. Anteriorly and on vertical half the face and perioral parts show a distinct elongate scaly reticulation, strongest at sides above toruli, but hardly raised. Whole posterior aspect reticulate unraised. The slightly projecting truncate median clypeal lobe is just wider than the distance between the toruli. Antenna (Fig. 1, *c*) length 65 mm., scape (6 : 1) equal to pedicel, ring joints and first three funicular joints together. Pedicel (11 : 6), as long as two-fifths of the scape, or the fourth and fifth funicular joints together; all the funicular joints transverse. Funicle practically cylindrical, club (5 : 4 : 4) hardly wider than funicle and as long as the last four joints of the latter. The major sensoria are about as long as the joints.

Labrum, simple transverse, edge sub-concave; bristles 2, 2. Mandibles (Fig. 1, *d*) (9 : 5) flat and elongate. The apical edge unusually oblique; the three upper teeth sub-equal. Trophi, stipes 5 bristles. Max. palp 4 : 6 : 9 : 17. The width increases from 4 to 6½. First two joints bare, third, 1 bristle, fourth, 1 lateral, 4 apical bristles.

Lab. palp 7 : 4 : 10, the last joint with 3–4 short bristles.

Thorax and propodeon (Fig. 1, *b*). Pronotum shining along the hind edge, but elsewhere with rather strong well-defined pattern (not shown in figure). Mesonotum entirely shining, the pattern on mesoscutum and parapsides not affecting the surface. *Scutellum entirely smooth* and *glassy*, broadly truncate posteriorly. Prepectora broadly fused—the median (ventral) area smooth. Pattern on sides weak. Mesosternum smooth, sternopleura dull, pattern raised, mesepisternite nearly quite smooth and very shining.

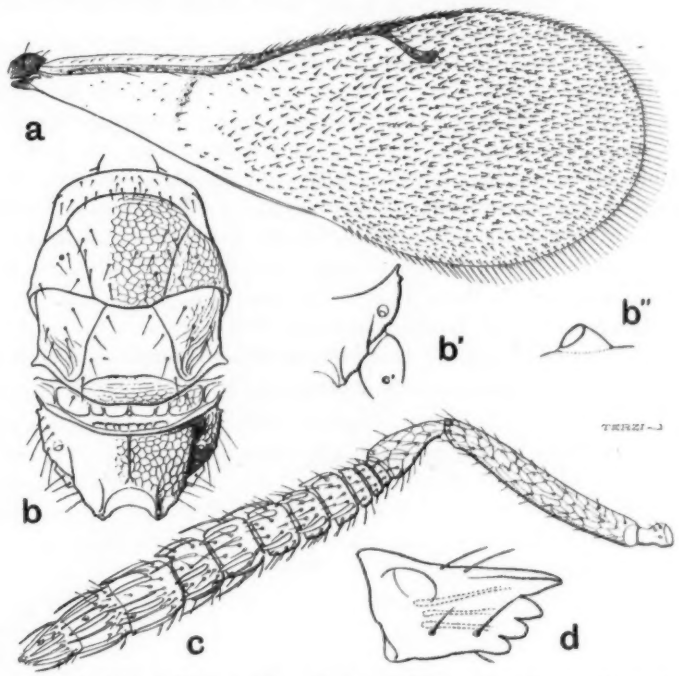

Fig. 1. *Bairamlia fuscipes* Waterston ♀ ; *a*, forewing; *b*, dorsum of thorax; *b'*, attachment of hind coxa (*c'*); *b''*, detail of spiracle; *c*, antenna; *d*, mandible.

Propodeon dull, pattern strongly raised. The only carina is the fine median one. The surface is dimpled on each side of the nucha; there is a larger depression in front of the slightly projecting spiracle behind which the surface falls rapidly to the insertion of the hind coxa. Metasternum smooth, the coxae inserted just behind the middle at sides.

Wings. Forewings (Fig. 1, *a*) 1 = 1·1 mm., two and a quarter times as long as broad; sub-marginal: marginal: radius: post-marginal 28 : 23 : 9 : 12.

Legs. Fore legs, coxa (11 : 6), large faint pattern. Femur (4 : 1). Tibia (7 : 2), comb of first tarsal joint 8–9 short spines: 6–7 more on plantar aspect of same joint. Tarsus 26 : 14 : 12 : 10 : 21. Mid legs coxa (4 : 3). Femur

(4 : 1). Tibia (13 : 2). Tarsus 26 : 20 : 13 : 11 : 20. Hind legs, coxa (3 : 2), pattern raised. Femur (22 : 5). Tibia (8 : 1). Tibial comb 8–10 spines. Spurs short; the longer barely one-half the first tarsal joint, which bears about 12 plantar spines. Tarsus 38 : 22 : 17 : 12 : 22.

Abdomen. Length of visible tergites approximately 3, 2, 1, 1, 2. At base of first (third) tergite a small truncate triangular heavily chitinised area giving out short rugae from its sides; on same tergite a trace of pattern and a patch of about 20 short stiff bristles on the overlaps anteriorly. Disc of abdomen smooth and bare except for a single row of short bristles (6, 6) on the tergites.

Spiracles small and nearly circular.

Length about 1·4 mm. Expanse about 2·5 mm.

The differences between the sexes are extremely slight. In the male the antenna is rather more slender with relatively shorter club, longer funicle, and broader scape. The mesonotal sculpture is a trifle coarser, the petiole just perceptibly longer and the propodeon with a slightly narrower nucha, *i.e.* the sides more strongly convergent. Dimensions about the same as in female.

Holotype ♀, allotype ♂ and paratypes (♂, ♀) in British Museum, from a small series bred from cocoons of *Ceratophyllus wickhami* Baker, taken from a squirrel's nest, Gerrard's Cross, Bucks, England (E. K. Sikes coll.).

♂, ♀, paratypes in U.S. National Museum, Washington.

Ceratophyllus wickhami, the flea of the introduced American Grey Squirrel (*Sciurus carolinensis* Gmel.), is now common in the London district.

(*MS. received for publication* 14, I. 1929.—Ed.)

TROCHOPUS GAILLIMHE[1] N.SP., AN ECTOPARASITIC TREMATODE OF *TRIGLA HIRUNDO* OR *TRIGLA LUCERNA*.

By PERCY A. LITTLE, B.Sc.

(*Late Acting Professor of Natural History at the National University College of Ireland, Galway.*)

(With Plates IX—XI and 8 Text-figures.)

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Introduction.

The Trematode herein described was obtained from the gills of *Trigla hirundo* Day, popularly called the Yellow Gurnard, Latchet or Tub, one of the most common species of the Gurnard family found on the west coast of Ireland.

It is an active Trematode, locomotion being effected by the alternating attachment and detachment of the anterior and posterior suckers whilst on the gills of its host coupled with the extension and contraction of its body.

The worms may live for many hours, even days, after removal from the host's gills provided the sea water is frequently changed and the temperature of the room remains low.

External Appearance.

Living specimens appear flat (Text-fig. 1), milky-white with a faint roseate tinge and are translucent, approaching transparency in the extended condition. The largest specimen of the lot is 5·85 mm. long. The anterior end bears a pair of well-developed suckers. In a specimen 5·03 mm. long the width was 2·78 mm. or five-ninths the body-length excluding the posterior sucker; this proportion appeared tolerably constant. The breadth of the body decreases slightly towards its posterior end. The posterior border is broad and bluntly rounded, having a slight concavity in its medial region where it is attached by a short peduncle to a conspicuously large posterior sucker. The convexity of all three suckers is directed dorsalwards.

[1] Gaillimh is the Gaelic name for Galway and pronounced as "Golliveh," accent on first syllable.

Two pairs of "eyes" (Text-fig. 1 (*e.*)) are situated dorsally at the anterior end of the body, one pair behind the other. They are situated on either side of the medial line and the posterior pair is slightly anterior to the ventrally placed mouth. The anterior pair are 0·14 mm. apart and 0·44 mm. from the anterior border of the body; they are smaller than the posterior pair which lie 0·28 mm. apart.

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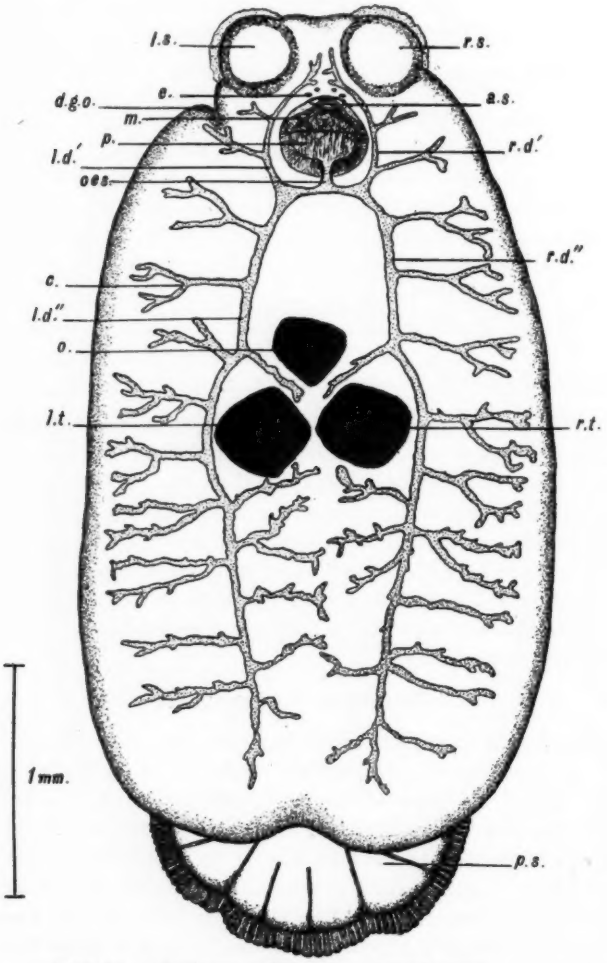


Text-fig. 1. *Trochopus gaillimhe* n.sp. Dorsal aspect.
a.s. aperture of sheath; *c.* dichotomously branched caecum; *d.g.o.* depression for genital openings; *e.* "eyes"; *l.d.'* left anterior diverticulum; *l.d."* left posterior diverticulum; *l.s.* left anterior sucker; *l.t.* left testis; *m.* mouth; *o.* ovary; *oes.* oesophagus; *p.* pharynx; *p.s.* posterior sucker; *r.d.'* right anterior diverticulum; *r.d."* right posterior diverticulum; *r.s.* right anterior sucker; *r.t.* right testis.

The *anterior suckers* (Text-figs. 1 and 2; also Pl. IX, fig. 2) are sessiled and have the appearance of deep saucers when viewed ventrally. Each sucker consists of two distinct zones: (*a*) an outer delicate semi-transparent membraneous frill, in which are numerous small darkly coloured mucous glands (*m.g.*) arranged in radially directed rows; (*b*) an inner firmer zone, whose tissues are largely muscular. These suckers are circular, their diameter varying from 0·45 mm. to 0·50 mm. Zone (*a*) is liable to severe shrinkage after treatment with fixing solutions, therefore measurements of this zone are not given. The musculature of zone (*b*) is highly developed, consisting of dorsal and ventral systems of concentrically arranged muscle fibres and numerous dorso-ventral muscle fibres.

The *posterior sucker* (Pl. X, fig. 5) is circular and 1·8 mm. in diameter. There are ten main rays: four anterior rays forming one group, three postero-lateral rays on each side constituting two additional groups. All three groups of rays converge towards the central region of the disc where they form a "horse-shoe" whose open end is directed posteriorly. The ten main rays extend from the base of a membraneous frill to the central region of the disc. In the space between the two posterior main rays (*p.r.*) is an additional pair of supplementary rays (*s.r.*) which do not extend much beyond a quarter of the diameter of the disc. The possession of ten main rays plus two supplementary rays distinguishes our species from other species of *Trochopus* hitherto described, with the exception of *T. lineatus* Scott which it resembles in this particular feature. Attached to the periphery of the posterior sucker is a delicate scalloped pleated frill; when viewed dorsally the posterior border of each small flap comprising the frill is slightly overlapped by the anterior border of the flap immediately behind, except in the region of the supplementary rays where overlapping of the posterior eight or ten flaps does not occur. Three pairs of hooks (*a*, *b*, *c*) occur in the posterior sucker of our species. *T. gaillimhe* differs therefore from *T. lineatus* Scott which has but two pairs (see Scott, Monticelli and Massa).

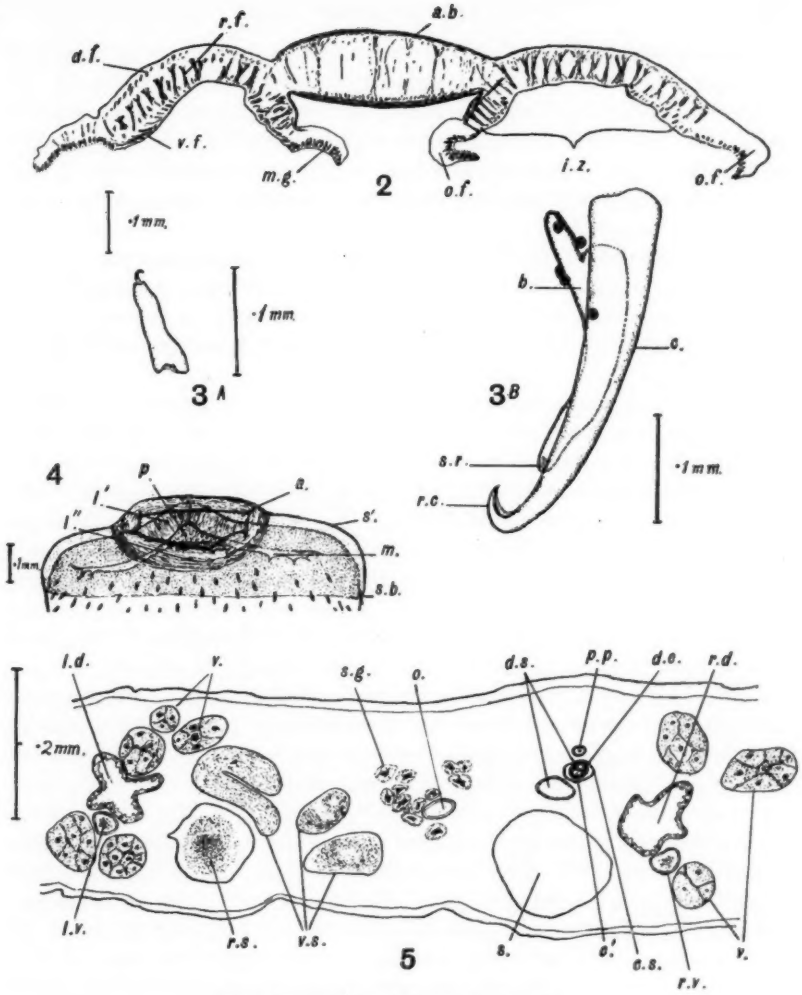
The *first pair of hooks* (Text-fig. 3 A and Pl. X, fig. 5 (*a*)) is situated at the junction of the three postero-lateral main rays and is directed towards the centre of the sucker. They are 0·1 mm. long, the larger part consisting of a cylindrical finger-shaped piece bearing a minute recurved claw apically.

The *second and third pairs of hooks* (Text-fig. 3 B (*b*, *c*)) are much stouter than the first pair and measure 0·30–0·35 mm. The second pair is situated on the inside and very close to the third pair. Towards the proximal end of the second pair are four or five condylar processes which serve for the attachment of muscles. At the distal end are situated four or five rows of serrated ridges (*s.r.*) resembling a file or rasp. It is probable that the second pair of hooks function by being pressed against the third pair which are opposite. The file-like apex increases their hold. The distal end of the third pair is sharply recurved, making an efficient claw (*r.c.*) whose apex can be seen protruding at the distal end of the posterior main ray (Pl. X, fig. 5 (*c*)). By far the

greater part of the shaft of the second and third pairs of hooks is embedded in the tissues of the posterior pair of main rays. Two well-developed groups of muscle fibres are intimately associated with each main shaft (Pl. IX, fig. 3): (*a*) those attached to the second pair may be regarded as adductors (*ad.*) (bringing the clamp-like hook into contact with the third pair) and the abductors (*abd.*); (*b*) those connected with the third pair act as flexors (*f.*) and extensors (*e.'*) respectively, the latter pulling the hook so as to disengage it from the tissues of the host.

Internal Anatomy.

The *alimentary system* (Text-fig. 1) is very diffuse and extends from the anterior to the posterior region of the body. The mouth (Text-fig. 4 (*m.*)) is situated ventrally 0·75 mm. from the anterior border of the body. It consists of a narrow transverse slit 0·60 mm. wide, its medial portion assuming a triangular-shaped orifice. The mouth is bordered by two prominent lips, the anterior (*l.'*) overlapping the posterior (*l.''*). The projecting lips and wide mouth are protected by a dome-shaped sheath (*s.*), the base of which is 0·82 mm. wide. The anteriorly directed apex of the sheath terminates in an oval aperture (*a.*) 0·30 mm. wide and 0·11 mm. long. The aperture of the sheath is situated 0·28 mm. from its base and is placed slightly anterior to the true mouth. Numerous muscle fibres occur in the sheath, those at its anterior end run parallel with its aperture, while posteriorly they are arranged along the longitudinal axis of the sheath. By virtue of the arrangement of the muscle fibres in the anterior end, the size of the aperture is controlled as by a sphincter muscle. The posteriorly disposed longitudinal muscle fibres can retract and extend the sheath so as to expose, or cover, the two prominent lips bordering the wide mouth. The mouth opens into a large oval pharynx (Pl. IX, fig. 1; also Text-fig. 1 (*p.*)), measuring 0·78 mm. × 0·40 mm. Numerous multicellular papillate structures (*pap.*) arise from all sides and project into the pharyngeal cavity. The apices of the papillae are distended with a granular substance (*g.'*) which is secretory and probably prevents the coagulation of blood obtained from the host's gills, or facilitates its extraction. Near the basal region of the papillae are numerous circular muscle fibres (*c.f.'*), while external to these are numerous muscle fibres radially directed (*r.f.*). The whole pharyngeal tissues are surrounded by a continuous layer of columnar epithelial cells (*e.*) (also Pl. X, fig. 6), beyond which is the general groundwork of parenchymatous tissue. Within the pharyngeal tissues are a few prominent nuclei. The oesophagus (Text-fig. 1 (*oes.*)) is short, 0·10 mm. long and 0·05 mm. wide. According to Massa (1906), *Trochopus onchacanthus* Massa and other species of the genus studied by him have no oesophagus. The oesophagus leads into the intestine which immediately bifurcates to form two intestinal branches which pass transversely left and right for a short distance. Each transverse branch gives rise to an anterior short diverticulum and a posterior diverticulum which extends to the posterior region of the body. The right and



Text-figs. 2–5. *Trochopus gaillimhe* n.sp.

Text-fig. 2. Section through body in region of anterior suckers.

Text-fig. 3 A. Ventral view of the right hook of the 1st pair.

Text-fig. 3 B. Ventral view of the left hooks of the 2nd and 3rd pairs.

Text-fig. 4. Ventral view of mouth.

Text-fig. 5. Transverse section of body a little anterior to the vitelline reservoir. Posterior aspect.

a. aperture of sheath; *a.b.* anterior end of body; *b.* second pair of hooks; *c.* third pair of hooks; *c.'* cirrus; *c.s.* cirrus sheath; *d.c.* ductus ejaculatorius; *d.f.* dorsal muscle fibres; *d.s.* duct from accessory sac; *i.z.* inner muscular zone; *l.'* anterior lip; *l.''* posterior lip; *l.d.* left posterior diverticulum: *l.v.* left anterior vitelline duct; *m.* mouth; *m.g.* mucous glands; *o.* oviduct; *o.f.* outer membraneous frill; *p.* papillae; *p.p.* pars prostatica; *r.c.* recurved claw; *r.d.* right posterior diverticulum; *r.f.* radial muscle fibres; *r.s.* receptaculum seminis; *r.v.* right anterior vitelline duct; *s.* accessory sac; *s.'* sheath; *s.b.* sheath base; *s.g.* shell gland; *s.r.* serrated ridges; *v.* vitelline glands; *v.f.* ventral muscle fibres; *v.s.* vesicula seminalis.

left anterior diverticula (*r.d.'*, *l.d.'*) remain close to the sides of the pharynx and terminate at a point midway between the anterior end of the body and the mouth. Each anterior diverticulum gives rise to two caeca which tend to branch dichotomously. Each posterior diverticulum (*r.d."*, *l.d."*) gives rise to a number of transversely directed branched caeca. Amoeboid cells (Pl. XI, fig. 9 (*a.c.*)) with prominent nuclei are found lining the lumen of the caeca and diverticula.

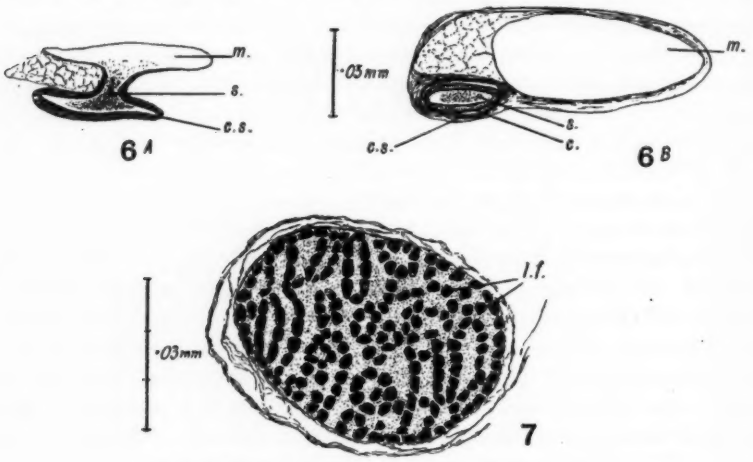
Reproductive system (Pl. X, fig. 4). The two genital openings are situated on the ventral surface, being placed sub-marginally and opening into a common depression located immediately posterior to the left anterior sucker. The genital openings are closely approximated and appear one behind the other.

Male system. The testes (*l.t.*, *r.t.*) are a pair of spherical organs of practically the same size, varying in diameter (0·45 mm.–0·55 mm.) in unpressed specimens, and 0·28 mm. thick. The inner surfaces of the testes almost touch, the organs running parallel; one of the testes may be slightly more anterior to the other. They lie midway along the length of the body, one on either side of the medial line. The two vasa deferentia originate midway along the inner border of each testis as figured, they pass anteriorly between the testes for a short distance and fuse to form the common vas deferens. The vas deferens (*v.d.*) descends abruptly to a point parallel with the ventral surfaces of the testes and running forward between the testes, turns slightly to the left, keeping close to the left testis, and passes between it and the ovary (*o.'*); it is a comparatively small duct and appears packed with sperm. It leads into a vesicula seminalis, entering it alongside its ventral and left surface. The vesicula seminalis (*v.s.*) is a capacious sac extending forward from the anterior margin of the left testis for a distance of about 0·60–0·70 mm., its diameter measuring 0·06–0·10 mm.; it twists about, as shown in Pl. X, fig. 4, along its whole course forming the letter "W" when viewed from the right side; it is packed with sperm throughout. It then becomes reduced to a small duct, the pars prostatica (*p.p.*), which turns to the right passing over the anterior end of the ootype and backwards whilst approaching the right side of the body; it then runs backward about 0·70–1·00 mm., close to the dorsal surface of the cirrus and when in a line with the anterior part of the ovary it turns about and enters the cirrus. The cirrus (*cir.*) originates at a point almost parallel with the anterior border of the vitelline reservoir (*v.r.*), or even as far back as the anterior border of the ovary; it runs forward, passing obliquely to the left side of the body and finally enters the metraterm ventrally in its last quarter length. The cirrus becomes looped about a third of its length from its origin. The length of the coils is such that if straightened out the apex of the cirrus would protrude beyond the genital opening. The cirrus (Pl. XI, fig. 11) is circular in cross-section, 0·06 mm. basally and tapering gradually anteriorly; it is enclosed in a sheath (*c.s.*) consisting of small squamous epithelial cells resting on the thin cuticle (*c.'*) lining the cirrus cavity. The cirrus has a strong

armature of cuticle encasing it (*c.''*). Traversing the length of the cirrus are two distinct ducts, each lined with cuticle (*c.'''*, *c.''''*). The larger duct is the continuation of the pars prostatica and may be regarded as the ductus ejaculatorius (*d.e.*); it is packed with sperm (*s.'*) along its course. The smaller duct (*d.s.*), which contains a gelatinous substance (*g.s.*), is connected to a large sac situated ventrally to the pars prostatica and to the cirrus (Text-fig. 5 (*s.*)). The occurrence of an accessory sac in Trematoda does not seem to have been hitherto observed. Massa (1906), in his description of *T. onchacanthus*, mentions the presence of a sac in about the same position but considers it to be a spermatic vesicle. The accessory sac in our species cannot be regarded as the vesicula seminalis for that organ has already been described as appearing on the left side of the body, and originating at the distal end of the vas deferens. Furthermore, the pars prostatica, which may be regarded as the continuation of the vesicula seminalis, whose diameter has become greatly constricted, passes without any discontinuity into the cirrus as the ductus ejaculatorius. Moreover, the accessory sac gives rise to a duct which enters the cirrus independently of the pars prostatica, remaining distinct from the ductus ejaculatorius which it accompanies throughout almost the entire length of the cirrus, becoming merged into one duct only at the apical region of the cirrus. Lastly, the contents of the vesicula seminalis and of the accessory sac react to stains differently. The accessory sac (Pl. X, fig. 4 (*s.*)) is 0·45–0·60 mm. long, its anterior margin being either parallel with, or slightly anterior or posterior to the junction of the oviduct and ootype. Its posterior margin is approximately parallel with the anterior border of the vitelline reservoir. It lies to the right of the medial line and ventral to the pars prostatica and cirrus. Its lumen, which is oval in outline, becomes suddenly enlarged anteriorly attaining to a width of 0·20–0·22 mm. Dorsally, within the anterior fourth of its length it gives rise to a duct having a diameter of 0·07 mm. at its origin; the accessory sac has a diameter of 0·14 mm. Thereafter the accessory sac maintains a fairly uniform diameter to its posterior blind termination. The duct, however, gradually diminishes in size as it runs backward, measuring 0·02 mm. when it abruptly turns forward to enter the cirrus, within which it remains distinct from the ductus ejaculatorius. The accessory sac is imbedded in glandular tissue. Both accessory sac and duct appear to contain the same gelatinous-like substance. In view of the habitat of *T. gaillimhe* upon the fish's gills—respiratory organs over which a constant current of water flows—it appears probable that the incorporation of a gelatinous substance with the sperm may ensure a better transference of the latter from the cirrus into the vagina.

Female system (Pl. X, fig. 4). The ovary (*o.'*) is spherical, 0·40 mm. in diameter and is situated in the region of the medial line, immediately in front of the testes, its posterior part often occupying the space anterior and between the testes. The oviduct (*od.*), emerging from the antero-dorsal surface of the ovary, almost immediately receives the unpaired common vitelline duct (*c.v.*)

from the prominent vitelline reservoir (*v.r.*), likewise the sperm duct (*s.d.*) from the receptaculum seminis (*r.s.*). The oviduct then passes anteriorly in a convoluted manner downward for a distance of about 0·50–0·60 mm. to the middle of the body. Its lumen is lined with irregularly shaped cells (Pl. XI, fig. 10) with large nuclei, but these cells disappear at its distal region where the lumen acquires a cuticular lining, in which condition it joins the ootype. Along its course the oviduct (Pl. X, fig. 4 (*od.*)) is surrounded by the cells of the shell gland (*s.g.*). The oviduct then joins the ootype (*o.*) entering its postero-ventral surface on the right side. The ootype is oval in outline with a maximum width of 0·11–0·12 mm.; its lumen is approximately triangular in shape (Pl. XI, fig. 12) owing to glandular tissue which is not uniformly



Text-figs. 6 A, 6 B, 7. *Trochopus gaillimhe* n.sp.

Text-fig. 6 A. Section through union of cirrus sheath and metraterm. Anterior view.
Text-fig. 6 B. Section immediately posterior to section 6 A. Anterior view.
Text-fig. 7. Section through a muscular zone in the region of the peduncle.
c. cirrus; *c.s.* cirrus sheath; *l.f.* longitudinal muscle fibres; *m.* metraterm; *s.* sperm.

developed around the lumen, but becomes constricted at three points. Large nuclei with prominent nucleoli are distributed throughout the tissue. A very thin layer of cuticle (*cu.*) lines the lumen of the ootype (Pl. XI, fig. 13). At its anterior end the ootype passes into the metraterm which is about 0·60–0·80 mm. long. The metraterm (Pl. X, fig. 4 (*m.*)) passes obliquely to the left and keeps parallel with the vagina (*v.*) which is situated immediately on its left. The metraterm terminates anteriorly in the anterior genital opening (*c.g.*). The metraterm receives the cirrus sac along its ventral surface (Text-fig. 6 A) at a point about one-fourth of its length from the genital opening. The cirrus does not occupy the cirrus sac at the actual point of union with the metraterm, the apical region of the cirrus being situated slightly posterior (Text-fig. 6 B).

In section, the cirrus sac is seen to be packed with sperm at its junction with the metraterm, into which the sperm appears to pass.

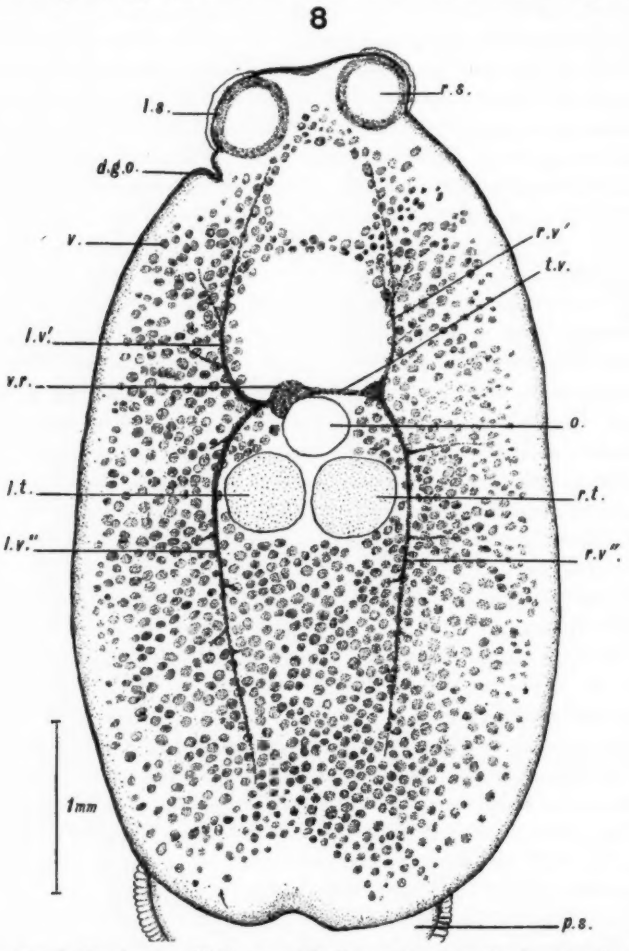
The mature eggs are pear-shaped, large, measuring 0·163–0·197 mm. by 0·100–0·142 mm. The shell is dark yellow, a twisted slender filament 0·386 mm. long being attached to the posterior end. Not more than one mature egg has been found in any one individual. The mature egg is situated either in the ootype with its filament occupying the oviduct or at the most posterior region of the metraterm, into which it appears to have been recently passed by the ootype. The vagina (Pl. X, fig. 4 (*v.*)) is 0·90–1·16 mm. long, passing obliquely inwards as it proceeds backward. The vagina, at first narrow, gradually widens to 0·05 mm. at a point slightly anterior to the ootype; it then narrows gradually to 0·02 mm. Peripheral longitudinal muscle fibres (Pl. X, fig. 7 (*l.f.*)) appear in this constricted portion of the vagina which again widens gradually to 0·06 mm. and becomes more muscular (Pl. X, fig. 8); longitudinal muscle fibres become more numerous and circular muscle fibres (*c.f.*) appear. The vagina becomes abruptly constricted where it joins the receptaculum seminis, this region being devoid of muscles. The receptaculum seminis (Pl. X, fig. 4 (*r.s.*)) is circular or oval in outline (0·17 × 0·17–0·14 mm.). The vagina and the receptaculum seminis remain in the ventral regions of the body throughout their entire course. In a transverse section the posterior termination of the receptaculum seminis has the appearance of being wedged between the dorsally situated vesicula seminalis and the ventrally placed vitelline reservoir. A sperm duct (*s.d.*) emerges from the posterior part of the receptaculum seminis, passes towards the medial line and enters the oviduct close to its union with the ovary.

The *vitelline glands* (Text-figs. 5 and 8 (*v.*)) consist of numerous follicles, circular to oval in outline (0·052–0·099 mm. × 0·052–0·082 mm.), lying dorsally and ventrally to the intestinal caeca. They are very voluminous, extending from the region immediately posterior to the anterior suckers to the hinder regions of the body close to its attachment to the peduncle. Their distribution is clearly shown in Text-fig. 8. A common unpaired vitelline duct (Pl. X, fig. 4 (*c.v.*)) emerges from the dorsal surface of the vitelline reservoir (*v.r.*) on the right side and approximately midway along its longitudinal axis and joins the oviduct close to its union with the ovary.

The *cells of the shell-gland* (Pl. XI, fig. 14) are large and possess conspicuous nuclei. Their extremely granular cytoplasm (*g.c.*) appears vacuolated. The product of their secretion accumulates to form dense irregularly shaped intracellular bodies (*i.b.*). Ducts from the shell-gland enter the oviduct at various points along its course.

Musculature of T. gaillimhe. The muscles of the anterior and posterior suckers have been dealt with, likewise those of the vagina. The musculature of the body wall (Pl. IX, fig. 1) consists of an outer zone comprising circular muscle fibres (*c.f.*) and an inner zone of longitudinal muscle fibres (*l.f.*), the fibres of the latter being arranged in groups of two, three, and four. Both

circular and longitudinal muscle fibres become more numerous as the posterior regions of the body are approached. In addition, oval areas of longitudinal muscle fibres (Text-fig. 7), situated in the mid dorso-ventral region of the



Text-fig. 8. *Trochopus gaillimhe* n.sp. Vitelline system, from the dorsal surface.

d.g.o. depression for genital openings; *l.s.* left anterior sucker; *l.t.* left testis; *l.v.'* left anterior vitelline duct; *l.v.''* left posterior vitelline duct; *o.* ovary; *p.s.* posterior sucker; *r.s.* right anterior sucker; *r.t.* right testis; *r.v.'* right anterior vitelline duct; *r.v.''* right posterior vitelline duct; *t.v.* transverse vitelline duct; *v.* vitellaria; *v.r.* vitelline reservoir.

body make their appearance towards the approach of the peduncle. As these areas of muscle fibres pass into and traverse the peduncle they decrease in number through fusion, and become larger in size, having an average width of 0·056 mm. Usually there are six such areas in the peduncle.

Systematic position and diagnostic characters.

The genus *Trochopus* was discovered by Diesing in 1850. In 1878 Taschenberg considered that the genus *Trochopus* should be treated as a species of the genus *Tristomum*. Monticelli in 1888 showed that the conclusions of Taschenberg could not be accepted and restored the genus *Trochopus* of Diesing. This restoration was upheld by Braun, St Remy, and all other authors who have worked on monogenetic Trematodes. Parona and Monticelli examined the species of the genus *Placunella* founded by Van Beneden and Hesse in 1863 for some Tristomes found on *Trigla* and *Rhombus* and concluded that the genera *Placunella* and *Trochopus* are synonyms, the latter holding priority. In 1906 Massa made a systematic revision of the genus and compiled a key to its species based upon their external characters.

Diagnostic characters of Trochopus gaillimhe *n.sp.*

Body. Milky-white with faint roseate tinge, translucent, 3·00–6·00 mm. long, flat, sub-rectangular.

Anterior suckers. Two, circular, sessile.

Posterior sucker. Circular, two-fifths to three-eighths length of body; delicate, marginal, scalloped, pleated frill; ten main and two supplementary rays; supplementary rays placed between the most posterior main rays; three pairs of hooks situated on the posterior pair main rays.

Genital openings. Open on ventral surface, submarginal, close behind the left anterior sucker.

Alimentary canal. Oesophagus short, diffuse system.

Testes. Two, spherical, situated midway along body-length, one on either side of the medial line.

Vesicula seminalis. Capacious, its course forming the letter "W."

Cirrus. Extends as far as genital opening.

Accessory sac. Large, connected with the emission of sperm.

Ovary. Spherical, situated on medial line, immediately anterior to testes.

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EXPLANATION OF PLATES IX—XI.

PLATE IX.

Trochopus gaillimhe n.sp.

Fig. 1. Transverse section through pharynx.
Fig. 2. Ventral aspect of anterior suckers.
Fig. 3. Transverse section through posterior sucker in the region of the posterior main ray. View, anterior aspect of right side.

PLATE X.

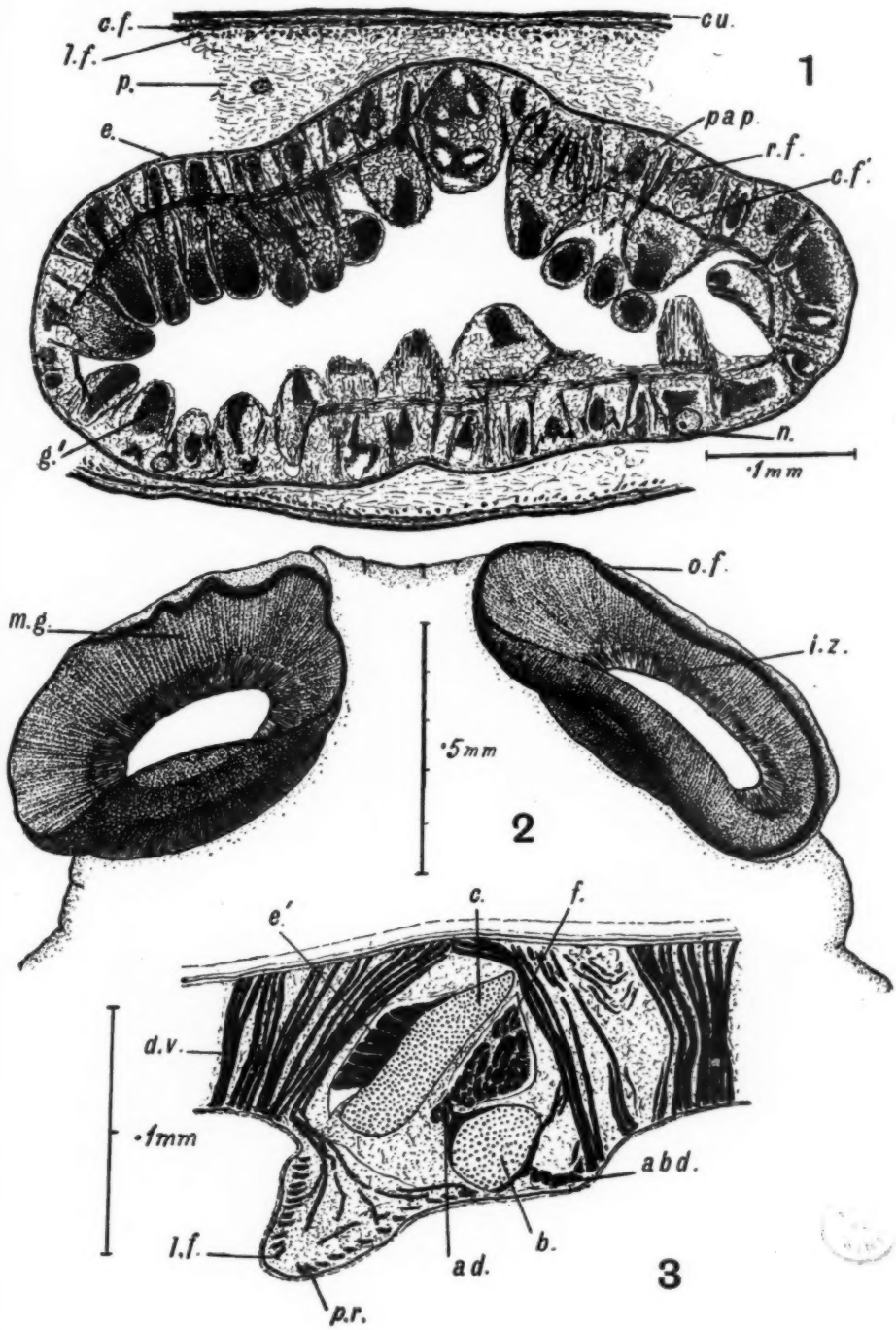
Trochopus gaillimhe n.sp.

Fig. 4. Schematic view of reproductive system. Dorsal aspect.
Fig. 5. Ventral aspect of posterior sucker.
Fig. 6. Columnar epithelial cells of the pharynx.
Fig. 7. Transverse section of vagina.
Fig. 8. Transverse section of vagina taken posteriorly to that figured in Fig. 7.

PLATE XI.

Trochopus gaillimhe n.sp.

Fig. 9. Digestive caecum in cross-section.
Fig. 10. Oviduct in cross-section.
Fig. 11. Cirrus in cross-section.
Fig. 12. Ootype in cross-section.
Fig. 13. Higher magnification of area marked "A" in Fig. 12.
Fig. 14. Cells of shell-gland.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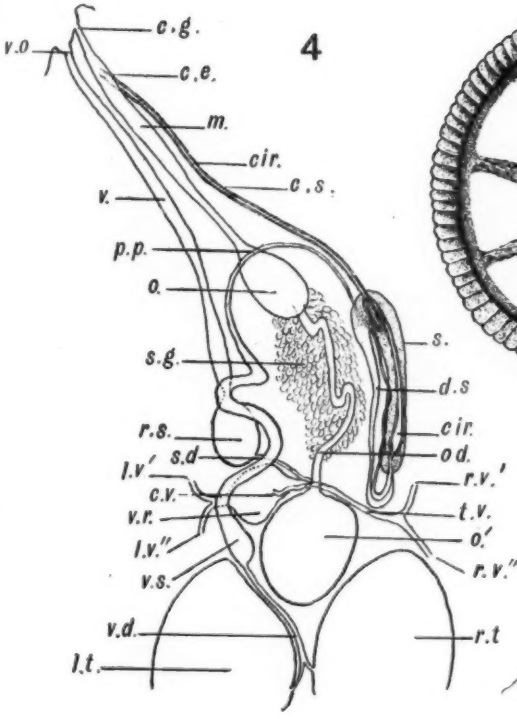
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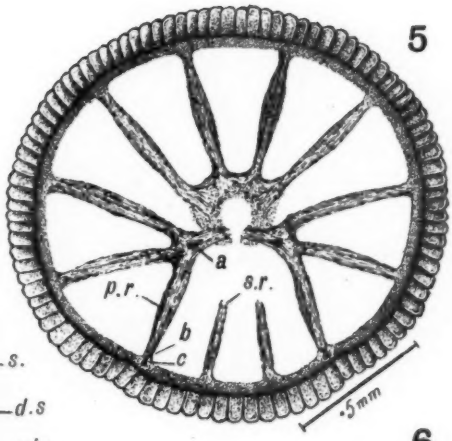
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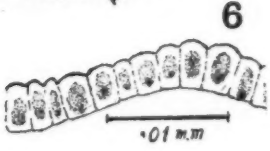
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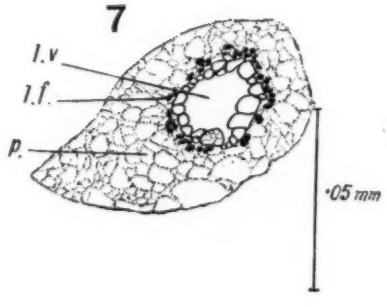
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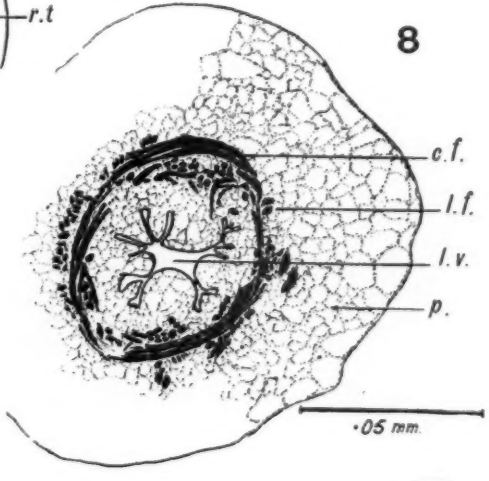
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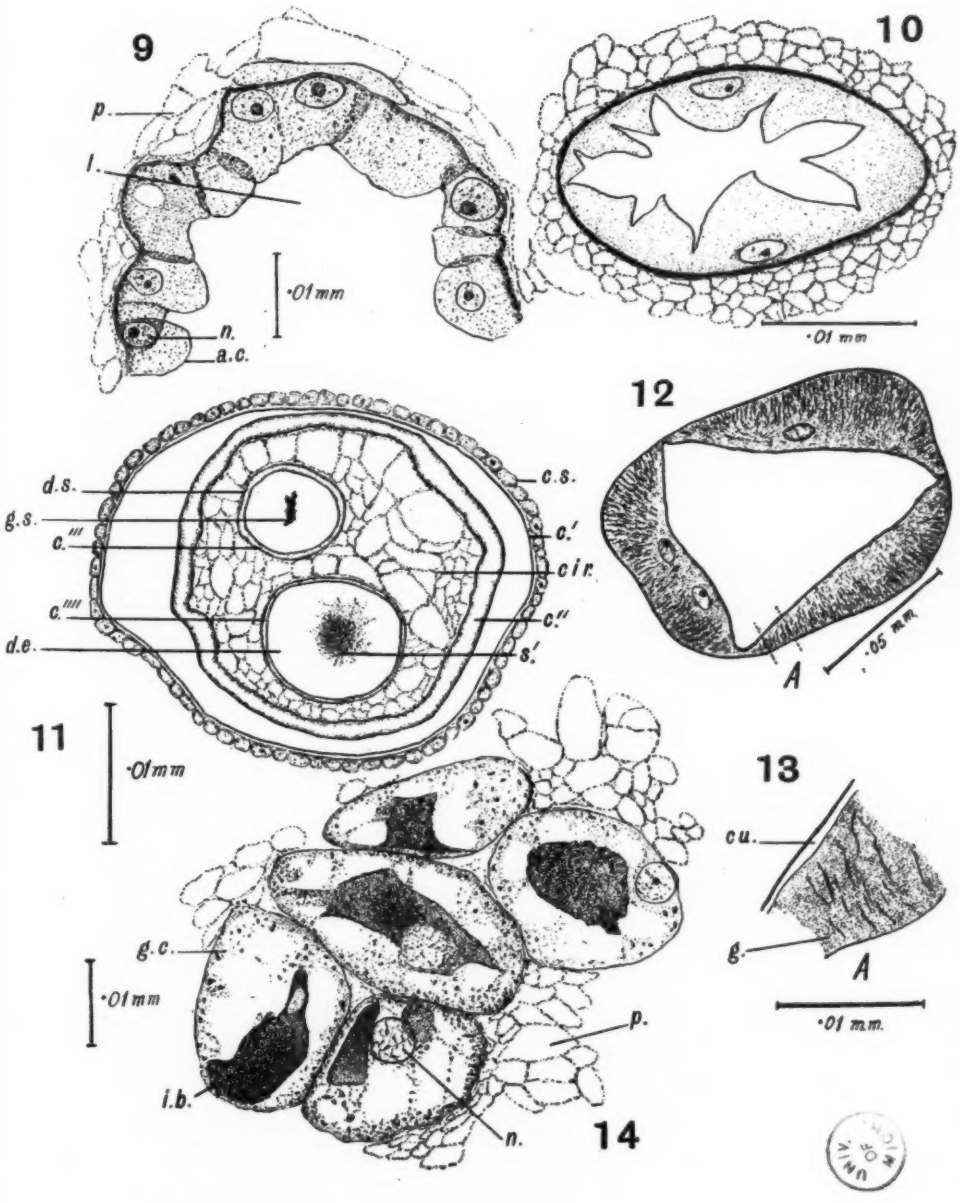
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KEY TO LETTERING OF PLATES IX—XI.

a. 1st pair of hooks
abd. abductor muscle
a.c. amoeboid cells
ad. adductor muscle
b. 2nd pair of hooks
c. 3rd pair of hooks
c.′, *c.″*, *c.‴*, *c.⁗* cuticle of cirrus
c.e. cirrus exit into metraterm
c.f. circular muscle fibres
c.f.′ circular muscle fibres of pharynx
c.g. common genital exit
cir. cirrus
c.s. cirrus sheath
cu. cuticle
c.v. common unpaired vitelline duct
d.e. ductus ejaculatorius
d.s. duct from accessory sac
d.v. dorso-ventral muscles
e. columnar epithelia
e.′ extensor muscles.
f. flexor muscles
g. glandular tissue
g.′ granular secretory substance
g.c. granular cytoplasm
g.s. gelatinous substance
i.b. intracellular body
i.z. inner muscular zone
l. lumen of caecum
l.f. longitudinal muscle fibres
.t. left testis
l.v. lumen of vagina
l.v.′ left anterior vitelline duct
l.v.″ left posterior vitelline duct
m. metraterm
m.g. mucous glands
n. nucleus
o. ootype
o.′ ovary
od. oviduct
o.f. outer membraneous frill
p. parenchyma
pap. papilla
p.p. pars prostatica
p.r. posterior main ray
r.f. radial muscle fibres of pharynx
r.s. receptaculum seminis
r.t. right testis
r.v.′ right anterior vitelline duct
r.v.″ right posterior vitelline duct
s. accessory sac
s.′ sperm
s.d. sperm duct
s.g. shell gland
s.r. supplementary ray
t v. transverse vitelline duct
v. vagina
v.d. vas deferens
v.o. external vaginal orifice
v.r. vitelline reservoir
v.s. vesicula seminalis

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ON THE DISTRIBUTION OF GREGARINES IN OLIGOCHAETES.

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Watson-Kamm (1916, 1922) has given complete lists of cephaline gregarine parasites from different classes of hosts, discovered up to 1920. She concluded that "except in rare instances a species is confined to a single host or to nearly related hosts, not being found in insects as far removed from each other as the Coleoptera and the Orthoptera." She believes there is an interesting parallelism between the complexity of the parasite and the group of hosts in which they are found. The simpler forms occurring in the Crustacea and Diplopodia, and the higher and more complex forms being found in various orders of Insecta. Many instances could be mentioned in which genera are found exclusively in a single order of insects.

In this paper, I propose to give a complete list of the acephaline gregarines parasitic in Oligochaetes and a list of the earthworm hosts with the parasites recorded from them. As shown in the lists, the genus *Aikinetocystis* is described from a single species occurring in four species of one genus. The genus *Stomatophora* includes five species which have been met with, both in Europe and in India, in the seminal vesicles of earthworms belonging to species of the genus *Pheretima* only. The closely related genera *Astrocystella*, *Craterocystis*, *Choanocystis*, *Choanocystoides*, *Beccaricystis*, and *Albertisella*, so far based on descriptions of single species, are also confined to the hosts of the genus *Pheretima*. It therefore appears justified to conclude that *Stomatophora* and allied genera of Monocystid parasites are phylogenetically no older than the host. The genera *Pleurocystis* and *Zygocystis* stand apart from the rest through the characteristic feature that in both the adult trophozoites are always found associated in pairs. These are confined to the genus *Allolobophora*, being found in the sub-genera *Helodrilus* and *Eisenia* only.

In *Rhynchocystis*, a genus characterised by the possession of a conical or cylindro-conical epimerite, we find *R. pilosa* confined to three species of *Lumbricus*; *R. porrecta* in the same three species of *Lumbricus* and in two species of *Allolobophora* (sub-genera *Eisenia* and *Helodrilus*); *R. piriformis* has been found in one species of *Allolobophora* (sub-genus *Eisenia*); *R. hessei* in two species of *Lumbricus* and in one species of *Pareudrilus* (belonging to the sub-family Eudrilinae of the family Megascolecidae). Of the species of *Rhynchocystis* described from India, *R. cognettii* has been found in *Allolobophora*

caliginosa, and two others viz., *R. mamillata* and *R. awatii* from *Pheretima elongata*. Thus, in the case of *Rhynchocystis*, species are known from worms belonging to *Lumbricus* and *Allolobophora* (family Lumbricidae), and *Pareudrilus* and *Pheretima* (family Megascolecidae). In the aberrant genus *Dirhynchocystis*, marked by the possession of a spine-like prolongation at either end, are included only two species, one described by Cognetti from *Fimoscolex inurus* (family Glossoscolecidae), and the other by Bhatia and Chatterjee from *Pheretima heterochaeta* (family Megascolecidae).

The genera *Monocystis*, *Apolocystis* and *Nematocystis* are the least differentiated among the Monocystids, possessing no epimerite or any other mark of differentiation, and in all of them we find species occurring in hosts belonging to different families. But as a rule any single species of parasite is confined to a single species or to two species of the same genus, or at the outside to closely related genera of hosts belonging to the same family. Thus among *Nematocystis* there are four species which are confined to hosts of the family Megascolecidae, five species confined to hosts belonging to the family Lumbricidae, and one to a host of the family Glossoscolecidae. *N. anguillula* is known from two species of *Pheretima*, *N. cecconi* from a species of *Pheretima*, *N. hessei* from a species of *Pheretima*, and *N. stephensoni* from a species of *Eutyphaeus*; on the other hand, *N. anguillula* var. *gracilis* has been recorded from three species of *Lumbricus*, *N. magna* from a single species of *Lumbricus*, *N. elongata* from a single species of *Octolasium*, *N. plurikaryosomata* from a single species of *Eisenia*, and *N. elmassiana* from a single species of each of the genera *Dendrobaena*, *Helodrilus* and *Lumbricus*. *N. almae* is confined to a single species of *Alma* belonging to the family Glossoscolecidae. Thus every one of the ten species of *Nematocystis* occurs in either a single species or two or more species of the same genus, or two or more allied genera belonging to the same family of hosts, and the same parasite has not been found to occur in hosts belonging to two different families. *N. vermicularis* would appear to form an apparent exception. It has been recorded by other writers from hosts belonging to one species of *Helodrilus* and two species of *Lumbricus*, but in 1925 Bhatia and Chatterjee recorded it from a species of *Pheretima*. On looking up this record, I find it is stated that in this last mentioned host "the infection is rather rare, as a single trophozoite has been found in one out of a large number of smears from the seminal vesicles of this worm." The size of the specimen again is much less than the usual size for that species. Our specimen may after all be a different species, or this particular slide may possibly be attributable to a different host owing to a confusion of labels.

Coming to the forms of a simple and more generalised type placed in the genera *Monocystis* and *Apolocystis* we find a more extensive distribution of the parasites among a number of families of hosts. Various species of *Monocystis* and *Apolocystis* are found in hosts belonging to all the families of earthworms examined so far, viz., Lumbriculidae, Enchytraeidae, Megascolecidae, Glossoscolecidae and Lumbricidae, but the same host-parasite specificity is also

found to occur here. The genus *Apolocystis* has been recognised by Cognetti to include spherical forms without any polar differentiation. Of the 8 species which may be referred to this genus, 5 are confined to hosts of the family Megascolecidae and 3 to hosts belonging to the family Lumbricidae. The former have been described from a species of *Pheretima*, *Megascolex*, or *Pareudrilus*, and of the latter, two are known from one species of *Octolasium* or of *Lumbricus*, and only one is known from two species of *Dendrobaena* and *Eisenia*, which are now regarded as sub-genera of *Allolobophora*.

Of the 43 species belonging to the genus *Monocystis* I have been unable to obtain particulars regarding three. Twenty are described from a single host species in every case, 7 from two or three species of hosts but belonging to the same genus, 11 from host species belonging to two different genera but belonging to the same family, and 2 only from host species belonging to 3 or 4 genera, but belonging to the same family.

Thus, judging from the data at present at our disposal the conclusion is reached that in the majority of cases a species of Monocystid parasites is confined to a single species of host, and where it does occur in more than one species of host, it is usual to find it restricted to two or more species of the same genus or two or more genera of the same family.

I. LIST OF MONOCYSTIDS WITH THEIR OLIGOCHAETE HOSTS.

Genus **Monocystis** Stein.

Parasite	Host	Seat
M. agilis Stein	*Lumbricus terrestris* L.	Vesiculae seminales
	L. rubellus Hoffm.	,,
	L. castaneus Sav.	,,
	Eisenia foetida Sav.	,,
M. perichaetae (Beddard)	*Diporochaeta intermedia* (Beddard)	,,
	Megascolex mauritii (Kinb.)	,,
M. lumbrici Henle	*Lumbricus rubellus* Hoffm.	,,
	L. castaneus Sav.	,,
	L. terrestris L.	,,
M. striata Hesse	*Lumbricus terrestris* L.	,,
M. hirsuta Hesse	*Lumbricus castaneus* Sav.	,,
M. crenulata Hesse	*Helodrilus* (*Allolobophora*) *longus* Ude	,,
	H. caliginosus var. *trapezoides* Ant. Dugès	,,
M. rostrata Mulsow	*Lumbricus terrestris* L.	,,
M. arcuata Boldt	*Lumbricus castaneus* Sav.	,,
	L. rubellus Hoffm.	,,
	Eisenia foetida Sav.	,,
M. mitis Leidy	*Fridericia polychaeta* Bretscher	Intestine
	Distichopus sylvestris Leidy	,,
M. cognettii Hesse	*Helodrilus chloroticus* Sav.	Vesiculae seminales
M. naidis von Voss	—	—
M. piriformis Boldt	*Octolasium complanatum* Rovigno	Vesiculae seminales and coelom

Parasite	Host	Seat
M. enchytraei Kölliker	*Enchytraeus albidus* Henle	Vesiculae seminales and coelom
	Fridericia galba (Hoffm.) = *Enchytraeus galba*	,,
	F. hegemon Vejd. = *Enchytraeus hegemon*	,,
M. pachydrili Clap.	*Lumbricillus pagenstecheri* Ratz.	Vesiculae seminales
	Marionina semifusca Clap.	,,
M. herculea Bosanquet	*Octolasium lacteum* Orley	Vesiculae seminales and coelom
	Lumbriculus terrestris L., Müll.	,,
	Lumbricus rubellus Hoffm.	,,
	L. castaneus Sav.	Vesiculae seminales
	Helodrilus caliginosus Sav.	,,
	H. chloroticus Sav.	,,
	Dendrobaena rubida var. *subrubicunda* Eisen	,,
M. lememei Hesse	*Helodrilus* (*Allolobophora*) *caliginosus* Sav.	Vesiculae seminales and coelom
M. bretscheri Hesse	*Fridericia polychaeta* Bretscher	Coelom
M. turbo Hesse	*Octolasium lacteum* Orley	Vesiculae seminales
M. turbo var. *suecica* Berlin	*Lumbricus rubellus* Hoffm.	,,
M. duboscqi Hesse	*Lumbriculus variegatus* (Müll.)	Vesiculae seminales and coelom
M. thamnodrili Cognetti	*Rhinodrilus* (*Thamnodrilus incertus*)	Coelom
M. perforans Pinto	*Glossoscolex wiengreeni* Mchlsn	Testes
M. bengalensis Ghosh	*Pheretima posthuma* (L. Vaill)	Vesiculae seminales
M. lloydi Ghosh	,, ,,	,,
M. beddardi Ghosh	*Eutyphaeus nicholsoni*	,,
M. pheretimi Bhatia and Chatterjee	*Pheretima posthuma* (L. Vaill)	,,
M. caudata Berlin	*Lumbricus rubellus* Hoffm.	,,
	L. castaneus Sav.	,,
	Helodrilus longus Ude	,,
M. densa Berlin	*Lumbricus terrestris* L.	,,
	L. rubellus Hoffm.	,,
	L. castaneus Sav.	,,
	Eisenia foetida Sav.	,,
	Helodrilus longus Ude	,,
	H. caliginosus (Sav.)	,,
	H. chloroticus (Sav.)	,,
M. securiformis Berlin	*Helodrilus caliginosus* (Sav.)	,,
M. ventrosa Berlin	*Lumbricus rubellus* Hoffm.	,,
(= *M. agilis forma b* Hesse)	*L. castaneus* Sav.	,,
	Eisenia foetida Sav.	,,
M. acuta Berlin	*Lumbricus rubellus* Hoffm.	,,
	L. castaneus Sav.	,,
M. hessei Berlin	*Lumbricus terrestris* L., Müll.	,,
	L. rubellus Hoffm.	,,
M. suecica Berlin	*Eisenia foetida* Sav.	,,
	Lumbricus terrestris L.	,,

Parasite	Host	Seat
M. wallengrenii Berlin	*Helodrilus longus* Ude	Vesiculae seminales and coelom
	H. caliginosus (Sav.)	,,
	Lumbricus rubellus Hoffm.	,,
M. polymorpha Berlin	*Lumbricus rubellus* Hoffm.	,,
M. tubiformis Berlin	*Lumbricus rubellus* Hoffm.	,,
	L. castaneus Sav.	,,
M. vivax Berlin	*Eiseniella tetraedra typica* Sav.	,,
M. carlgreni Berlin	*Lumbricus terrestris* L., Müll.	,,
	L. rubellus Hoffm.	,,
M. oblonga Berlin	*Lumbricus terrestris* L., Müll.	,,
	L. rubellus Hoffm.	,,
	L. castaneus Sav.	,,
	Helodrilus longus Ude	,,
M. ciliata Drzewecki	—	—
M. macrospora Hesse	*Pheretima rodericensis* Grube	Coelom
	Pheretima hawayana Rosa	,,
M. tricingulata Cognetti	*Pheretima* (*Parapheretima*) *beaufortii* Cogn.	Lymphatic glands

Genus **Apolocystis** Cognetti.

A. michaelseni (Hesse)	*Pheretima hawayana* Rosa	Coelom
A. villosa (Hesse)	*Octolasium lacteum* Orley	Vesiculae seminales
A. beauforti (Cognetti)	*Pheretima* (*Parapheretima*) *beauforti* Cogn.	,,
A. ambigua (Cognetti)	,,	Lymphatic glands
A. lumbrici olidi (Hesse)	*Dendrobaena rubida* Sav.	Vesiculae seminales
	D. rubida var. *subrubicunda* Eisen	,,
	Eisenia foetida Sav.	,,
A. pareudrili (Cognetti)	*Pareudrilus pallidus*	,,
A. matthai (Bhatia and Setna)	*Megascolex trilobatus*	,,
? *Monocystis catenata* Mulsow.	*Lumbricus terrestris*	Coelom

Genus **Nematocystis** Hesse.

N. vermicularis Hesse	*Helodrilus* (*Allolobophora*) *longus* Ude	Vesiculae seminales and coelom
	Pheretima barbadensis (Beddard)	Vesiculae seminales
	Lumbricus terrestris L., Müll.	,,
	L. rubellus Hoffm.	,,
N. anguillula Hesse	*Pheretima rodericensis* Grübe	,,
	P. hawayana (Rosa)	,,
N. anguillula var. *gracilis* Berlin	*Lumbricus terrestris* L., Müll.	,,
	L. rubellus Hoffm.	,,
	L. castaneus Sav.	,,
N. magna (Schmidt)	*Lumbricus terrestris* L., Müll.	Vesiculae seminales and lymphatic glands
N. lumbricoides Hesse	*Helodrilus caliginosus* Sav.	Vesiculae seminales
	Pheretima heterochaeta (Mchlsn)	,,

Parasite	Host	Seat
N. cecconii Cognetti	*Pheretima* (*Parapheretima*) *wendessiana* Cogn.	Coelom and lymphatic glands
N. almae Cognetti	*Alma emini* Mich. var. *aloysii-sabandiae* Cogn.	Coelom
N. elmassiani (Hesse)	*Dendrobaena rubida* var. *subrubicunda* Eisen	Vesiculae seminales
	Helodrilus (*Allolobophora*) *longus* Ude	Coelom
	Lumbricus terrestris L., Müll.	Vesiculae seminales
N. elongata Hesse	*Octolasium lacteum* Orley	Ciliated pavilion
N. hessei Bhatia and Chatterjee	*Pheretima heterochaeta* (Mchlsn)	Vesiculae seminales
N. plurikaryosomata Bhatia and Chatterjee	*Allolobophora* (*Eisenia*) *foetida* (Sav.)	,,
N. stephensoni Bhatia and Setna	*Eutyphoeus incommodus* (Beddard)	,,
Genus **Rhabdocystis** Boldt.		
R. claviformis Boldt	*Octolasium complanatum* Rovigno	,,
Genus **Rhynchocystis** Hesse.		
R. pilosa (Cuénot)	*Lumbricus terrestris* L., Müll.	,,
	L. rubellus Hoffm.	,,
	L. castaneus Sav.	,,
R. porrecta (Schmidt)	*Lumbricus terrestris* L., Müll.	,,
	L. rubellus Hoffm.	,,
	L. castaneus Sav.	,,
	Allolobophora (*Eisenia*) *foetida* Sav.	,,
	Helodrilus caliginosus	,,
R. hessei Cognetti	*Pareudrilus pallidus*	,,
	Lumbricus terrestris L., Müll.	,,
	L. rubellus Hoffm.	,,
R. piriformis Berlin	*Allolobophora* (*Eisenia*) *foetida* (Sav.)	,,
R. cognettii Bhatia and Chatterjee	*Allolobophora caliginosa* (Sav.)	,,
R. mamillata Bhatia and Setna	*Pheretima elongata* (E. Perr.)	,,
R. awatii Bhatia and Setna	,,	,,
Genus **Dirhynchocystis** Cognetti.		
D. brasiliensis Cogn.	*Fimoscolex inurus* Cogn.	,,
D. globosa (Bhatia and Chatterjee)	*Pheretima heterochaeta* (Mchlsn)	,,
Genus **Stomatophera** Drzewecki.		
S. coronata (Hesse)	*Pheretima rodericensis* (Grube)	,,
	P. hawayana (Rosa)	,,
	P. barbadensis (Beddard)	,,
S. borealis Drzewecki	—	,,
S. diadema Hesse	*P. hawayana* (Rosa)	,,
	P. barbadensis (Beddard)	,,
S. simplex Bhatia	*P. rodericensis* (Grube)	,,
S. bulbifera Bhatia and Setna	*P. elongata* (E. Perr.)	,,

Parasite	Host	Seat
Genus **Grayallia** Setna.		
G. quadrispina Setna	*Pheretima heterochaeta* (Mchlsn)	Vesiculae seminales
Genus **Astrocystella** Cognetti.		
A. lobesa Cogn.	*Pheretima* (*Parapheretima*) *beaufortii* Cogn.	,,
Genus **Craterocystis** Cognetti.		
C. papua Cogn.	*Pheretima* (*Parapheretima*) *wendessiana* Cogn.	Prostate gland and lymphatic glands
Genus **Choanocystis** Cognetti.		
C. tentaculata Cogn.	*Pheretima* (*Parapheretima*) *beaufortii* Cogn.	Vesiculae seminales and capsule seminales
Genus **Choanocystoides** Cognetti.		
C. costaricensis Cogn.	*Pheretima heterochaeta* (Mchlsn)	Vesiculae seminales
Genus **Beccaricystis** Cognetti.		
B. loriai Cogn.	*Pheretima* (*Parapheretima*) *sermowaiana* Cogn.	,,
Genus **Albertisella** Cognetti.		
A. crater Cogn.	*Pheretima* (*Parapheretima*) *sermowaiana* Cogn.	,,
Genus **Pleurocystis** Hesse.		
P. cuenoti (Hesse)	*Helodrilus* (*Allolobophora*) *longus* Ude	Pavillon cilié
	H. (*A.*) *caliginosus* Sav.	,,
Genus **Zygocystis** Stein.		
Z. cometa Stein	*Lumbriculus agricola* Hoffm.	Coelomic cavity and vesiculae seminales
	Helodrilus (*Allolobophora*) *longus* Ude	Vesiculae seminales
	H. (*Allolobophora*) *caliginosus* Sav.	,,
	H. caliginosus var. *trapezides* Ant. Dugès	,,
	H. chloroticus Sav.	,,
Z. pilosa (Hesse)	*Helodrilus* (*Allolobophora*) *longus* Ude	,,
Z. legeri (Hesse)	*Helodrilus chloroticus* Sav.	,,
Z. suecica Berlin	*Eisenia foetida* Sav.	,,
Genus **Aikinetocystis** Gates.		
A. singularis Gates	*Eutyphoeus foveatus*	Coelomic cavities
	E. spinulosus	,,
	E. rarus	,,
	E. peguanus	,,

II. LIST OF OLIGOCHAETE HOSTS AND THEIR MONOCYSTID PARASITES.

Family LUMBRICULIDAE.

Host	Parasite	Seat
Genus **Lumbriculus** Grube.		
L. variegatus (Müll.)	*Monocystis duboscqi*	Vesiculae seminales
Family ENCHYTRAEIDAE.		
Genus **Fridericia** Mchlsn		
F. polychaeta Bretscher	*Monocystis mitis* Leidy	Intestine
	M. bretscheri	Coelom

Host	Parasite	Seat
F. galba (Hoffm.)	*M. enchytraei* Kölliker	Vesiculae seminales and coelom
F. hegemon Vejd	,,	,,
Genus **Enchytraeus** Henle.		
E. albidus Henle	*M. enchytraei* Kölliker	,,
Genus **Distichopus** Leidy.		
D. sylvestris Leidy	*Monocystis mitis* Leidy	Intestine
Genus **Marionina** Mchlsn.		
M. semifusca Clap.	*Monocystis pachydrili* Clap.	Vesiculae seminales
Genus **Lumbricillus** Örst.		
L. pagenstecheri Ratz	*Monocystis pachydrili* Clap.	,,
Family MEGASCOLECIDAE.		
Sub-family MEGASCOLECINAE.		
Genus **Megascolex** Templeton.		
M. mauritii (Kimb.)	*M. perichaetae* (Beddard)	Vesiculae seminales
M. trilobatus (Steph.)	*Apolocystis matthai* (Bhatia and Setna)	,,
Genus **Pheretima** Kimb.		
P. barbadensis (Beddard)	*Nematocystis vermicularis* Hesse	,,
	Stomatophora coronata (Hesse)	,,
	S. diadema Hesse	,,
P. beaufortii Cogn.	*Monocystis tricingulata* Cogn.	Lymphatic glands
	Apolocystis beauforti (Cogn.)	Vesiculae seminales
	A. ambigua (Cogn.)	Lymphatic glands
	Astrocystella lobosa Cogn.	Vesiculae seminales
	Choanocystis tentaculata Cogn.	Vesiculae seminales and capsule seminales
P. hawayana (Rosa)	*Monocystis macrospora* Hesse	Coelom
	Apolocystis michaelseni (Hesse)	,,
	Nematocystis anguillula Hesse	Vesiculae seminales
	Stomatophora coronata (Hesse)	,,
	S. diadema Hesse	,,
P. heterochaeta (Mchlsn)	*Nematocystis lumbricoides* Hesse	,,
	N. hessei Bhatai and Chatterjee	,,
	Dirhynchocystis globosa (Bhatia and Chatterjee)	,,
	Choanocystoides costaricensis Cogn.	,,
	Grayallia quadrispina Setna	,,
P. elongata (E. Perr.)	*Rhynchocystis mamillata* Bhatia and Setna	,,
	R. awatii Bhatia and Setna	,,
	Stomatophora bulbifera Bhatia and Setna	,,
P. posthuma (L. Vaill)	*Monocystis bengalensis* Ghosh	,,
	M. lloydi Ghosh	,,
	M. pheretimi Bhatia and Chatterjee	,,
P. rodericensis (Grube)	*Monocystis macrospora* Hesse	Coelom
	Nematocystis anguillula Hesse	Vesiculae seminales

Host	Parasite	Seat
	Stomatophora coronata Hesse	Vesiculae seminales
	S. simplex Bhatia	,,
P. (*Parapheretima*) *sermowaiana* Cogn.	*Beccaricystis loriai* Cogn.	,,
	Albertisella crater Cogn.	,,
P. wendessiana Cogn.	*Nematocystis cecconi* Cogn.	Coelom and lymphatic glands
	Craterocystis papua Cogn.	Prostate gland and lymphatic glands
Genus **Diporochaeta** Beddard.		
D. intermedia (Bedd.)	*Monocystis perichaetae* (Bedd.)	Vesiculae seminales
	Sub-family EUDRILINAE.	
Genus **Pareudrilus** Beddard.		
P. pallidus	*Apolocystis pareudrili* Cogn.	,,
	Rhynchocystis hessei Cogn.	,,
	Sub-family OCTOCHAETINAE.	
Genus **Eutyphoeus** Mchlsn.		
E. nicholsoni (Bedd.)	*Monocystis beddardi* Ghosh	,,
E. incommodus (Bedd.)	*Nematocystis stephensoni* Bhatia and Setna	,,
E. foveatus	*Aikinetocystis singularis* Gates	Coelomic cavities
E. spinulosus	,, ,,	,,
E. rarus	,, ,,	,,
E. peguanus	,, ,,	,,
	Family GLOSSOSCOLECIDAE.	
Genus **Glossoscolex** Leuck.		
G. wiengreeni (Mchlsn)	*Monocystis perforans* Pinto	Testes
Genus **Rhinodrilus** Perrier.		
R. (*Thamnodrilus*) *incertus*	*Monocystis thamnodrili* Cogn.	Coelom
Genus **Fimoscolex** Mchlsn.		
F. inurus Cogn.	*Dirhynchocystis brasiliensis* Cogn.	Vesiculae seminales
Genus **Alma** Grube.		
A. emini var. *aloysii-sabandiae* Cogn.	*Nematocystis almae* Cogn.	Coelom
	Family LUMBRICIDAE.	
Genus **Allolobophora** Eisen.		
A. (*Eisenia*) *foetida* (Sav.)	*Apolocystis lumbrici olidi* (Hesse)	Vesiculae seminales
	Monocystis agilis Stein	,,
	M. arcuata Boldt	,,
	M. suecica Berlin	,,
	M. densa Berlin	,,
	M. ventrosa Berlin	,,
	Nematocystis plurikaryosomata Bhatia and Chatterjee	,,
	Rhynchocystis porrecta (Schmidt)	,,
	R. piriformis Berlin	,,
	Zygocystis suecica Berlin	,,
A. caliginosa (Sav.)	*Monocystis lememei* Hesse	Vesiculae seminales and Coelom

Host	Parasite	Seat
A. caliginosa (Sav.)	*M. densa* Berlin	Vesiculae seminales
	M. wallengrenii Berlin	,,
	M. herculea Bosanquet	,,
	Nematocystis lumbricoides Hesse	,,
	Rhynchocystis porrecta (Schmidt)	,,
	R. cognetti Bhatia and Chatterjee	,,
	Pleurocystis cuenoti (Hesse)	Pavillon cilié
	Zygocystis cometa Stein	Vesiculae seminales
A. caliginosa var. *trapezoides* (Ant. Dug.)	*Monocystis crenulata* Hesse	,,
	Zygocystis cometa Stein	,,
A. longus Ude	*Monocystis crenulata* Hesse	,,
	M. caudata Berlin	,,
	M. densa Berlin	,,
	M. wallengrenii Berlin	,,
	M. oblonga Berlin	,,
	Nematocystis elmassiani (Hesse)	,,
	N. vermicularis Hesse	Vesiculae seminales and coelom
	Pleurocystis cuenoti (Hesse)	Pavillon cilié
	Zygocystis cometa Stein	Vesiculae seminales
	Z. pilosa (Hesse)	,,
A. chloroticus (Sav.)	*Monocystis cognettii* Hesse	,,
	M. densa Berlin	,,
	M. securiformis Berlin	,,
	M. herculea Bosanquet	,,
	Zygocystis cometa Stein	,,
	Z. legeri (Hesse)	,,
A. (*Dendrobaena*) *rubida* (Sav.)	*Apolocystis lumbrici olidi* (Hesse)	,,
A. (*Dendrobaena*) *rubida* var. *subrubicunda* Eisen	*Apolocystis lumbrici olidi* (Hesse)	,,
	Monocystis herculea Bosanquet	,,
	Nematocystis elmassiani (Hesse)	,,
Genus **Eiseniella**.		
E. tetraedra typica Sav.	*Monocystis vivax* Berlin	,,
Genus **Octolasium** Orley.		
O. complanatum Rovigno	*Monocystis piriformis* Boldt	,,
	Rhabdocystis claviformis Boldt	,,
O. lacteum Orley	*Monocystis herculae* Bosanquet	,,
	M. turbo Hesse	,,
	Apolocystis villosa (Hesse)	,,
	Nematocystis elongata Hesse	Pavillon cilié
Genus **Lumbricus** L., Eisen.		
L. terrestris L., Müll.	*Monocystis agilis* Stein	Vesiculae seminales
	M. lumbrici Henle	,,
	M. striata Hesse	,,
	M. rostrata Mulsow	,,
	M. herculea Bosanquet	,,
	M. suecica Berlin	,,

Host	Parasite	Seat
L. terrestris L., Müll.	*M. densa* Berlin	Vesiculae seminales
	M. hessei Berlin	,,
	M. carlgrenii Berlin	,,
	M. oblonga Berlin	,,
	M. catenata Mulsow	Coelom
	Nematocystis vermicularis Hesse	Vesiculae seminales
	N. anguillula var. *gracilis* Berlin	,,
	N. amagna (Schmidt)	,,
	N. elmassiani (Hesse)	,,
	Rhynchocystis pilosa (Guénot)	,,
	R. porrecta (Schmidt)	,,
	R. hessei Cognetti	,,
L. rubellus Hoffm.	*Monocystis agilis* Stein	,,
	M. lumbrici Henle	,,
	M. arcuata Boldt	,,
	M. herculea Bosanquet	,,
	M. turbo var. *suecica* Berlin	,,
	M. tubiformis Berlin	,,
	M. caudatus Berlin	,,
	M. densa Berlin	,,
	M. ventrosa Berlin	,,
	M. acuta Berlin	,,
	M. hessei Berlin	,,
	M. wallengrenii Berlin	,,
	M. polymorpha Berlin	,,
	M. carlgrenii Berlin	,,
	M. oblonga Berlin	,,
	Nematocystis vermicularis Hesse	,,
	N. anguillula var. *gracilis* Berlin	,,
	N. elmassiani (Hesse)	,,
	Rhynchocystis pilosa (Cuénot)	,,
	R. porrecta (Schmidt)	,,
	R. hessei Cognetti	,,
L. castaneus Sav.	*Monocystis agilis* Stein	,,
	M. lumbrici Henle	,,
	M. hirsuta Hesse	,,
	M. arcuata Boldt	,,
	M. herculea Bosanquet	,,
	M. tubiformis Berlin	,,
	M. caudatus Berlin	,,
	M. densa Berlin	,,
	M. ventrosa Berlin	,,
	M. acuta Berlin	,,
	M. oblonga Berlin	,,
	Nematocystis anguillula var. *gracilis* Berlin	,,
	Rhynchocystis pilosa (Cuénot)	,,
	R. porrecta (Schmidt)	,,
L. agricola Hoffm.	*Zygocystis cometa*	Coelomic cavity and vesiculae seminales

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THE SIGNIFICANCE OF THE *p*H IN THE DEVELOPMENT OF MOSQUITO LARVAE.

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(With 1 Text-figure.)

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1. Introduction.

In 1921, during work on the bionomics of the British tree-hole breeding mosquitoes, I observed that the addition of tap water to the jars containing larvae in the laboratory resulted either in the death of the larvae or in the inhibition of their normal development (1921, *Parasitology*, **13**, pp. 348–351). Investigation disclosed the fact that the cause of this phenomenon was in some way correlated with the reaction of the water, and was sufficiently defined to be determined by the use of *p*H indicators. My conclusions then were, "While I would not go so far as to say, at present, that the abnormal development of mosquito larvae in the laboratory is entirely due to changes in the hydrogen-ion exponent (concentration), it, nevertheless, has a profound influence upon the metabolism of the larvae, and their resistance to diseases which affect them."

These observations met with considerable attention from workers in many parts of the world, and both confirmatory and conflicting evidence on this hypothesis has now been brought forward. Between the years 1921 and 1926 I have had many opportunities for extensive investigations, and although both the field and laboratory studies made during this period have clearly indicated that the explanation of the phenomenon is very difficult to acquire, I have still at least some reason to feel that the *p*H index is often a valuable index of the conditions in the breeding-place which portend the successful or non-successful development of mosquito larvae.

The fact that larvae of certain species are invariably to be found in waters of a short *p*H range, and that some species under natural conditions may be classed as acidophile and alkalinophile species with reference to the reaction of the water they normally inhabit, here points to the probability that the *p*H index is also an index of the suitability or otherwise of the particular waters for the successful development of the larvae of the particular species. On the other hand, it seems equally true from the work of numerous investigators, whose reports are confirmed by my own previous or subsequent observations, that certain species of mosquito larvae are to be found in waters showing a fairly extensive *p*H range.

The evidence which among certain species so consistently would prove that the *p*H index is correlated with successful or unsuccessful larval development is thus at times conflicted by evidence which seems to prove the opposite in regard to other species. Consequently, of late, many workers have turned to a search for some other factors which will explain why the larvae of certain species are only to be found in particular breeding-places, and why they do not inhabit indiscriminately all kinds of natural and artificial breeding-places in one or more localities. We are thus now familiar with the terms "dissolved and residual oxygen," "the nitrogen ratio," "the larval food-supply," "phytoplankton," etc., yet in reality the influence of none of these things alone seems even as trustworthy as is the *p*H index in some cases.

As concerns my own observations, there are four clearly established facts which the present work has taken into consideration: (1) Two of the British Anophelines (*Anopheles maculipennis* and *A. bifurcatus*) are found invariably in waters which have either a neutral or an alkaline reaction; while the third British species, *A. plumbeus*, under natural conditions is only found in the temporarily neutral or permanently acid water of tree holes. (2) Acid waters in the immediate neighbourhood of the breeding-places of *A. maculipennis* and *A. bifurcatus* are never found to be inhabited by the larvae of either of these species. (3) It is possible to tell which of two larval culture media (originally similarly composed but subsequently having acquired different reactions) will permit and which will preclude successful larval development. (4) That the mere artificial reversal of the *p*H index is often sufficient to determine the fate of the larvae. In these four instances the larval development is correlated with the *p*H index.

Keilin (1927) has reported the discovery of water in a horse chestnut tree hole at Cambridge having a *p*H of 9·1. This high *p*H index is certainly abnormal.

It has been said that the only acceptable evidence which would prove the *p*H to have any correlation with the breeding-places of mosquitoes must be of a statistical nature, wherein, by thousands of records, it shall be determined, for example, that Anopheline larvae are found in the natural waters whose *p*H index is in the alkaline range. Yet statistical evidence of this sort can quite easily be misleading. For instance, out of 1000 records in certain localities 900 might indicate alkalinity in the water, 99 might indicate acidity, and 1 might indicate neutrality. If only in the last Anopheline larvae were found, the statistical evidence, *per se*, would indicate that the Anophelines were not to be found either in acid or alkaline waters, but quite probably such a conclusion would be entirely wrong. The "type" of natural breeding-place is of paramount importance, and the "types" of breeding-places show wide and definite specific differences. Perfectly convincing as statistical records would be if they were taken in the full light of field experience, purely statistical method is dangerous and is apt to induce on the part of the impressionable the acceptance of dogmatic mathematical calculations as an easy and comfortable solution of the problem under investigation.

From the results of the different investigations here recorded, it is, I think, now possible to form a fairly clear conception of the significance of the *p*H in its correlation with the suitability or unsuitability of the breeding-places to particular species of mosquito larvae. In the first place we can now safely say that the hydrogen-ion concentration, *per se*, has little, if any, direct effect upon the larvae themselves, since although it is true that the larvae of *Aëdes argenteus* and certain other species develop best under natural conditions in water with an alkaline reaction, and that in the majority of cases these larvae are found in such waters, it is possible to show experimentally under particular circumstances that the larvae will develop satisfactorily in waters which are either alkaline, neutral, or comparatively strongly acid.

Nevertheless, although we may state that the larvae under particular circumstances will develop satisfactorily either in acid or alkaline water, it would seem in the case of larvae of *Aëdes argenteus* that the optimum natural development in all instances occurs in alkaline water. However, many of our experiments appear to prove that the reaction of the water is of some direct importance to the larvae when food is absent. It has been shown, for instance, that larvae of *Aëdes argenteus* in pure distilled water will survive longer when the water is alkaline than when it is acid. Then again, the almost invariable rule with larvae in bread media, etc., which is at first alkaline and may subsequently become acid through fermentation, is that the larvae do well only while the alkaline reaction persists, and die rapidly when acidity ensues. It has been demonstrated, moreover, that if this acid medium is allowed to revert naturally to a condition of alkalinity once more, or is made alkaline by the

addition of ammonia, that the larvae can resume successful development even in the previously fatal environment.

Such evidence induces, *prima facie*, the natural conviction that the precise reaction of the water is of definite importance to the larvae. But evidence to the contrary is shown strikingly in the case of larvae under aseptic conditions; where we discover, in the case of larvae raised under bacteriologically sterile conditions, that the reaction of even the bread media plays little or no part in influencing their successful development, for under these conditions the media may be made definitely acid without adversely affecting the larvae.

From these facts we are consequently inclined to ask whether the *p*H after all can have any useful significance? The field observations which support the hypothesis "that there is often some correlation between the *p*H index and the presence or absence of certain species of larvae" cannot, however, be lightly brushed aside, and long experience has taught us that with most species of mosquito larvae we undoubtedly often get a reliable index under natural conditions as to the suitability or unsuitability of particular waters for the development of the larvae of particular species by the *p*H index. Even in the laboratory we can differentiate between jars of media by means of the *p*H indicators, as to which may be used for raising the larvae successfully, and which may not.

On what interpretation of experimental and field observation is this apparently conflicting evidence to be reconciled? The underlying truth seems to be that the development of mosquito larvae under natural conditions is dependent not upon one, but upon many factors, and that it is idle to search for or to lay stress on the importance of any particular factor. The very existence of one factor is often dependent solely on the presence or absence of another. The successful development of mosquito larvae rests on the complicated equilibrium of chemical and biological interactions. Fundamentally it is the chemical constitution of the waters which determines the presence of what may be destined to become the food supply of the larvae. In general the ultimate food supply depends upon the activities of micro-organisms either by their multiplication on elements valueless to the larvae themselves, or by the conversion or decomposition by micro-organisms of plant and animal tissues. Biological competition ensues in a favourable environment, antagonism between groups prevails, and the success or failure of the self-preservation of any group depends upon its control and dominance of its antagonists. This swinging conflict of biological activity obviously tends to change, or does in effect change, the actual chemical composition of the water, lending thus from time to time either sustained or temporary support to one or other of the conflicting groups. We may define the resultant chemical condition of the water in terms of "dissolved oxygen," "residual oxygen," the "nitrification ratio," etc. as we prefer, but the matter of primary importance is that the stability of the system under natural conditions must result in most instances in a comparatively definite condition of the physico-chemical features of the water.

It is here that the significance of the *p*H index is disclosed. For whether the resultant condition of the chemical constitution of the water gives rise either to acidity or to alkalinity indicates in many cases whether the prevailing state there denotes a favourable or unfavourable environment for the larvae. However, this index is clearly not infallible, for as it has been shown experimentally it does not always provide satisfactory evidence. The most that can be said for the *p*H index in anti-mosquito work, therefore, is, that it frequently affords under natural conditions a reliable index also of the combination of the factors which connote the suitability or otherwise of particular waters for particular species. The more fully the general requirements of the larvae of particular species are studied, the more widely will it be recognised that these requirements are, in nature, representative of a definite set of conditions which frequently are reflected by the *p*H index.

2. Records.

Barber and Komp (1922), in investigations in Alabama, found that the *p*H index of natural waters in this State varied from *p*H 5 (ditch below springs) to *p*H 8 (overflow from artesian wells). They state that numerous Anophelines were found in both of these extremes, and in water of nearly every intermediate grade. As all the species of *Anopheles* common in this region (*Anopheles punctipennis*, *A. quadrimaculatus* and *A. crucians*) were found in waters of greatly varying *p*H, their view was that there appears to be no measurable correlation between the hydrogen-ion concentration of waters and these three species.

Williamson (1924), in the Federated Malay States, showed that the occurrence of particular species is correlated with a definite short *p*H range in the waters of the different breeding-places. In the rice-growing districts he observed the almost complete cessation of Anopheline breeding in standing rice when the water has a *p*H of 6 or lower with a sharp decrease below *p*H 6·4. He states that in that locality the conditions suitable for Anopheline breeding are, in general, the same as those producing a good crop of rice: acid and peaty soils restrict both the species and their abundance, only *A. barbirostris* Wulp, and *A. hyrcanus* var. *sinensis* Wied. being present. Nevertheless, other observations made show that abundant breeding of some of the same species that are found in the rice crop may occur in water of a much greater degree of acidity than is favourable to them in the rice fields, provided that there is a plentiful supply of food, and that other conditions, as yet not fully known, are favourable. An insufficiency of food is thought to be more important as a cause of death among nearly fully grown larvae than any direct influence exerted by the water of the breeding-places.

Darling (1925), in Georgia, stated that investigations of the hydrogen-ion concentration of waters showed that a larger proportion of *A. crucians* is taken from waters which are sub-acid, while *A. punctipennis* prefers waters which are very slightly alkaline. *A. quadrimaculatus*, he claimed, is mostly found in waters more nearly neutral.

A note by Brumpt (1925) in Catanei's paper (1925) is to the effect that *A. bifurcatus* in Corsica cannot exist in water which has a *p*H index lower than 5·4–5·2. At the higher altitudes of the island the alkaline waters of the plains become neutral or even acid, and the distribution of the species is to be accounted for to some extent on the significance of the *p*H index.

Morishita (1925) affirms that the hydrogen-ion concentration of the breeding-places of *Culex fatigans* near Taikoku City is between 6·5 and 7·6. In a given locality it does not alter so greatly as to effect the fauna in a short period. Between *p*H 2·0 and 9·8 the hatching of the eggs takes place normally, although in a water of *p*H 2 the newly hatched larvae die soon afterwards. It is therefore concluded that the hatching itself is not influenced by the hydrogen-ion concentration of the water. Between *p*H 5·7 and 7·6 the newly hatched larvae survive a week even without food, and in this period *C. fatigans* can nearly complete its aquatic life, if food is available and other conditions are favourable. Between *p*H 5·3 and 9·4 when food is present, growth and metamorphosis proceed almost at the same rate, and there is no special preference for either acid or alkaline waters. It is therefore thought that in the case of *C. fatigans* the hydrogen-ion concentration of the environment is of little importance as a factor in development.

As a result of his observations Senior-White (1926) concludes that the natural waters of Ceylon extend in *p*H range from 5·4 to 9·2, but that mosquito larvae in general are only found between *p*H 5·8 and 8·6. Species living in moving water are said to have wider *p*H tolerance than those in standing water, and that the former extend further into acidity and the latter further into alkalinity. Anophelines are stated to have wider tolerances than Culicines.

Harold (1926) gives details of the hydrogen-ion concentration of water in which various species of mosquito larvae have been found in England under natural conditions. *Aëdes maculatus* Mg., *Aëdes annulipes*, *Aëdes geniculatus* and *Aëdes rusticus* are recorded as occurring in water with a *p*H of 4·7–4·9; *Aëdes punctor* and *Aëdes cinereus* invariably, and *Theobaldia morsitans* normally, occur in acid water although they were found in water of a *p*H up to 7·5; *Anopheles bifurcatus*, *Anopheles maculipennis* and *Theobaldia annulata* occur normally in alkaline water, but are stated to have been found in water with a *p*H of 6–6·8. Harold, however, considers that the hydrogen-ion concentration of waters is of minor importance and that the factor of prime importance is food.

Walton and Rees Wright (1926) deduced from experiments on the alteration of the hydrogen-ion concentration by the addition of acetic acid that the larvae of *Aëdes detritus*, *Culex pipiens* and *Anopheles maculipennis* were unaffected, and "remained alive until the water dried up."

Beklemishev and Metrofanova (1926), working in the region of the Kama River, state that the optimum conditions for the existence of the larvae of *Anopheles maculipennis* appear to be calm water, a depth of about 12–20 inches, direct sunlight, supersaturation with oxygen, neutral or weak alkalinity

(*p*H 7·1–7·95), and a rich submerged vegetation and fauna. The absence of suitable food is, in their opinion, apparently the direct factor which makes any particular piece of water unfit for the development of larvae, and that this is frequently influenced by a lack of dissolved oxygen or by an acid reaction of the water.

Nikitinshii (1926) finds that *Anopheles maculipennis* occurs in turf pits in which the water has a *p*H of 5·64 and that its numbers increase with the increase of *p*H. Under natural conditions the larvae apparently prefer phytoplankton to zooplankton as food. The waters with a *p*H below 5, such as occur in sphagnum peat, are poor in phytoplankton which is represented by one group only, *i.e.* the Desmidiaceae. He concludes that it is therefore possible that the physico-chemical factors do not act directly on the distribution of *Anopheles maculipennis*, but indirectly through their effect on the phytoplankton.

The observations of Buxton and Hopkins (1927) in Samoa on *Culex fatigans*, *Aëdes variegatus* var. *pseudoscutellaris* and *Aëdes argenteus* have led them to the conclusion that none of these three species is influenced by the *p*H of the water in choosing a place in which to lay eggs. Larvae of all three were found in water of from *p*H 7·0 to 8·1 or 8·2 and no apparently suitable breeding-places were found outside these limits. Experimental investigations made by these two investigators confirmed their opinion that the *p*H does not affect the successful development of the larvae. "But though the *p*H appears to exercise no effect within ordinary limits, there is evidence that very acid or very alkaline fluids are definitely unattractive to the female."

Barber (1927) states that *Anopheles quadrimaculatus* and *Anopheles crucians* are known to thrive in nature at a *p*H of between 6·5 and 8·5, but that in laboratory experiments the larvae have been found to do well at a concentration as low as *p*H 5 so long as the food supply and other conditions were favourable.

Hamlyn-Harris (1927), in Brisbane, reports that he has observed in the case of *Anopheles annulipes* that the *p*H of the breeding-places of this species seemed to show a definite toleration limit of *p*H 6·5–7·5, except in one case where the *p*H was 8, but concludes that food is the main determining factor in the selection of the breeding-places.

Scharff (1927), in Singapore, states from his observations that a large part of the matter ingested by Anopheline larvae in Malaya is completely disorganised and dead, and in his opinion the grading of species according to the quality of their breeding-waters seems to be more probably brought about by unsuitability of food, leading to food-poisoning, than by any other single cause. He concludes in accepting the hypothesis that pure-water species are kept from breeding in even slightly fouled water either by poisoning or infectious disease; that it seems more likely that the responsible factors are food-poisoning and food-infection than that they are changes in the hydrogen-ion concentration or problematically present poisonous nitrogenous bases in solution.

Russell (1927), working in Macedonia, has found that the Anophelines without exception breed only in waters having a *p*H of 8–9·5. "Whatever the factor in water which attracts Anophelines (be it the flora, fauna or a chemical factor) the *p*H reading is sufficiently often an index of its presence and so of Anopheline larvae, in the district under consideration to be of definite practical value to the anti-malaria worker." She states, however, that the local Culicines are not so selective and are to be found in natural waters giving readings from about *p*H 6·5 to 10.

Williamson (1928) states that the correlation between hydrogen-ion concentration and the larvae of certain Malayan Anophelines has been shown to differ in different types of water (Williamson, 1924), or, in other words, to have been dependent upon associated factors. Not only have various observers recorded wide ranges of *p*H for a number of Anopheline species, but also abundance of larvae has been found at widely differing extremes (Barber, 1922), a fact in agreement with Malayan records. If hydrogen-ion concentration directly and alone determined larval prevalence, maximal abundance should occur at about the middle of the natural range of a species. Among associated factors, micro-organisms, whether serving as larval food (Lamborn, 1922) or producing disease (MacGregor, 1921) are probably of primary importance.

Hamlyn-Harris (1928) with reference to *Culex fatigans*, states, "Its principal breeding-places as a rule have a *p*H ranging from 7·4 to 8·6, and one is inclined to believe that it is in this state of concentration [*p*H] that the food suited to the species is most likely to occur. If by experimentation, the *p*H is swung to acidity in the region of 5·0, the death of the larvae and papae soon follows."

The fact that the *p*H index in certain instances is often a significant index also, as to whether the larvae of particular species of mosquito will or will not develop successfully in certain waters, made it highly desirable that investigation should, if possible, show the exact influence which the *p*H exerted: in other words whether the acidity or alkalinity of the water has a direct influence upon the larvae themselves, or has an indirect influence by controlling other factors upon which larval development depends. On so large an organism as a mosquito larva any direct influence or minute differences in acid and alkaline concentrations, admittedly, seems unlikely. However, when it is remembered that a concentration of 0·000,004 HCl has the effect of instantly stopping the action of the excised heart of a frog, it is well to consider that the existence or death of all living tissue cells may be directly determined by the precise electromolecular condition of fluids in which such cells are bathed.

It is clear that if alterations in the *p*H index are brought about experimentally by the addition of alkalis or acids to the water the normal chemical content of the water is disturbed to some extent. For this reason means were sought which would bring about a change in the *p*H index without the addition to the water of extraneous acids or alkalis, and the use of an electric current for this

purpose was investigated. Unfortunately, while it was found that the *p*H of some waters was readily altered by this means, and that the larvae could tolerate a low voltage current at a density of approximately 10 milliamperes (MacGregor, 1926), it was observed that the electrolysis which led to the molecular dissociation of the solutes frequently resulted in the liberation of toxic radicles such as Cl, SO_2, H_2S, etc., and the interpretation of results in consequence was impossible. Electric methods had therefore to be abandoned.

As the investigations proceeded it became obvious that whatever influence the reaction of the medium had, that influence was to a great extent obscured by a host of other interacting factors. Such factors, for instance, as changes in the chemical constitution of the medium as it became older and was no longer subject to control under natural conditions by the influence which the normal bacterial flora exerted upon the larvae; either directly by the production of disease, by too high a concentration of toxic products, or indirectly, as a food supply to the larvae and as food-producing agents.

3. The effect of altering the *p*H index in the absence of a food supply.

It was first thought advisable to ascertain whether alterations in the *p*H influenced the period of survival of larvae in bacteriologically filtered marsh water. High-vacuum filtration of marsh water was effected *via* a Chamberland's F. bougie and batches of 25 1st instar larvae of *Aëdes punctor* (*nemorosus*) were placed in tubes of two groups. In the tubes of Group I the *p*H of the water was found to be *p*H 8·5, and the *p*H of the water in the tubes of Group II was reduced to *p*H 4 by the addition of dilute acetic acid. A striking result followed, since most of the larvae in the tubes of Group I lived from 7 to 15 days, and succeeded in growing to some extent, while all the larvae in the tubes of Group II were dead within 3 days. Any high concentration of bacteria and solid food materials had been eliminated in these experiments, and except for the possibility that the acid reaction of the water led to the development of some pathogenic organisms from the air which killed the larvae, we had here suggestive evidence that larvae normally flourishing in alkaline water cannot survive in acid water.

4. Investigations with *Aëdes argenteus*.

Owing to the time of year, to the influence of drought in causing the temporary disappearance of our local ponds and marshes, and to my realisation that in working with "wild" species [*Aëdes punctor* (*nemorosus*)] we were confronted with the influence of many unknown factors in its normal development, such as temperature, visible and ultra-violet light, etc., I determined to continue the work with a species more amenable to laboratory conditions. I therefore wrote to Herr Dr Prof. E. Martini of Hamburg by whose courtesy I have been able to establish a strain of *Aëdes argenteus* with which the subsequent investigations have been conducted.

The specimens received from Prof. Martini were despatched from Hamburg as dried eggs on blotting-paper, larvae in water contained in glass tubes, and others on damp moss housed in a small aluminium box. By an accident, the parcel was delayed in London *en route* to this laboratory for 5 days, and therefore the specimens were in transit from Hamburg for at least 7 days. Nevertheless, when the parcel was opened at this laboratory the majority of the larvae were alive and still healthy even though they had been tightly confined in their containers. The eggs and the larvae were immediately placed in tap water to which had been added fallen leaves and dried twigs, and a strain of *Aëdes argenteus* was thus successfully established.

Now it is interesting to observe that the study of the factors which govern development for which these larvae had been obtained, was called to further attention before the first batch had reached maturity. It has long been recognised that the rate of development of the larvae of *Aëdes argenteus* and other species is not uniform; some rapidly passing through their metamorphosis, while others generally pass through the larval stage slowly or even very slowly indeed. The several hundred dried eggs on the blotting-paper received from Prof. Martini were placed in a large glass vessel containing 5 litres of tap water and 500 grm. of dried leaves and twigs, at a constant temperature of 30° C. The eggs hatched in from 30 seconds to 1 day after having been placed in this vessel, and at the end of 24 hours the blotting-paper was removed. By that time the water held hundreds of 1st instar larvae, and 2 days later the majority had entered the second instar. From this time onwards, however, the rate of development of individual larvae was conspicuously different, for on the 16th day the first adult mosquitoes emerged, while there were still large numbers of 2nd–4th instar larvae in the vessel. The possibility of some of these larvae having arisen from eggs which had floated off the blotting-paper and delayed their hatching was considered for a time, but finally I had the 5th generation from the more rapidly emerged specimens side by side with larvae of the ancestral strain. Many of the latter remained as larvae from early June to late August 1927, perfectly healthy and active as far as could be judged, even slowly growing, but for some reason at a rate that was preposterously slow and exhibiting what may be termed the phase of "suspended development."

5. *p*H experiments on the larvae of *Aëdes argenteus*.

In the earliest of these experiments the "fallen-leaf, dried-twig and tap-water" medium was used. Observations were made on larvae at all instars, and the separate tubes employed in these experiments were divided into three groups: Group I contained larvae in this medium at the normal hydrogen-ion concentration (*p*H 8–8·5); Group II had the water adjusted to *p*H 4–4·5 by the addition of dilute acids; and the water of Group III was given a *p*H of 9·2–10 by the addition of dilute ammonia. It was found that the larvae in Group II either died rapidly or failed to continue their development satis-

factorily, while those of Groups I and III maintained their normal development. Again, however, the interpretation of results was clearly hopelessly obscure. Was the effect in the acid tubes directly due to a production of poisonous compounds, the destruction of bacterial species engaged upon the decomposition of the vegetable tissues from which the larvae ultimately obtained their food supplies, or did the acidity of the medium permit the growth of pathogenic organisms? The fact that there were still too many unknown factors to be reckoned with was again manifest, in spite of the fact that the temperature and light factors were now under control. Moreover, I had still to contend with the great variance in the rate of development of individual larvae.

6. Experiments to reduce the number of unknown factors controlling the development of *Aëdes argenteus*.

Taking into consideration the question whether the essential food elements of the larvae in the "leaf" medium might either be the macerated vegetable tissues, cultures of protozoa and bacteria growing on the dead tissues, or a combination of both, the production of which is unquestionably dependent upon the *p*H of the medium, the matter of primary importance for future work was the discovery of some already-made artificial foodstuff on which the larvae could be reared successfully. The following table gives records of the materials used and of their relative food value as judged by the development or non-development of larvae in specified time periods.

7. Artificial materials employed as larval food.

Table I.

Twenty-five larvae in tubes containing 50 c.c. of food media: temperature 30° C.

Material	*Culture medium*	*Remarks*
Pure corn starch	0·1 % in distilled water (*p*H 7·8)	Useless as a food. Dissection showed that the larvae had taken up large amounts, but the starch granules in the intestine were unchanged
Pure potato starch	0·1 % in distilled water (*p*H 7·8)	Similar
Boiled starch	0·1 % in distilled water (*p*H 8·0)	Of slight value. Possibly indirectly by bacterial and mould growth. Some larvae developed to the 2nd instar in 15 days; remainder died earlier
Cane sugar	0·1 % in distilled water (*p*H 7·4)	No value in itself as a food
Bacto-peptone	0·1 % in distilled water (*p*H 7·4)	Fairly satisfactory. Heavily contaminated with bacteria after 2 days. Some larvae completed metamorphosis slowly (21 days), but if medium became strongly acid (as frequently happened) larvae died rapidly
Casein	0·1–3 % in distilled water (*p*H 8·0)	Fairly satisfactory. Larvae develop slowly if strong acidity does not appear
Egg albumin	0·1–5 % in distilled water (*p*H 7·4)	Of variable value, according to the degree of bacterial and mould contamination

Table I (*continued*).

Material	*Culture medium*	*Remarks*
Egg yolk	0·1–3 % in distilled water (*p*H 7·4)	A good food at low concentration (0·1 %) if bacterial decomposition does not liberate H_2S. The frequent liberation of this gas leads to the rapid death of the larvae
"Bemax"	0·1–5 % in distilled water (*p*H 8·5)	An excellent food at low concentration (0·1–3 %) if the medium does not become acid
"Standard" bread	0·5 % in distilled water (*p*H 8·5)	Apparently contains all the necessary food elements for vigorous larval growth. Larvae grow to a magnificent size at a rapid and practically uniform rate. Metamorphosis usually complete in from 10 to 12 days in media which remains alkaline. Larvae die rapidly if marked acidity develops
"Brown" bread	0·5 % in distilled water (*p*H 8·5)	Similar
"White" (French) bread	0·5 % in distilled water (*p*H 8·5)	Almost similar, though possibly not quite so good
Boiled oatmeal	0·5 % in distilled water (*p*H 7·8)	Larvae developed satisfactorily, though rather slowly (18–25 days). Larvae in some tubes die suddenly when medium becomes strongly acid
Powdered beans (boiled)	0·5 % in distilled water (*p*H 8·0)	A fairly good food. Acidity in this medium apparently not so fatal a condition down to *p*H 6·5
Peter's synthetic medium	Normal concentration (*p*H 8·5)	Larvae grew very slowly to 3rd instar by the 18th day. Actual food probably bacterial contamination

In these experiments the following procedure was adopted. Fifty cubic centimetres of freshly distilled water at *p*H 7–7·5 were placed in separate glass tubes each provided with celophane-covered corks through which passed a short length of glass tubing plugged with cotton-wool to prevent gross bacterial contamination, and to exclude falling dust. To each of these tubes were added 20 1st instar larvae from a mixed batch of eggs which had been laid on distilled water by females kept in special captivity for the purpose. Previously weighed amounts of the food materials under test were then added, and the tubes closed by the special corks. A celophane covering was used to prevent any cork dust (a possible source of food) from falling into the tubes. All the tubes were then placed in an incubator well illuminated by daylight at a temperature of 30° C.

With experiments on the relative food values of different substances the greatest care is necessary that the actual substance and no other is available to the larvae. Such experiments call for perfect cleanliness in all glass vessels used as containers, and bacteriological methods in the careful handling of the experiments even when they are not conducted antiseptically. It is astonishing on how little organic matter larvae will often develop more or less successfully. Until this truth was learnt and the actual necessity of using chemically clean containers for the larvae was realised, our readings of results were often incorrect, simply from the fact that the larvae were obtaining, either from the

air or from the apparently clean glass walls of their tube-containers, organic matter sufficient to invalidate many of our observations. For instance, larvae of *Aëdes argenteus* in distilled water can obtain a small amount of nourishment from such substances as clean cotton-wool, and clean white filter-paper.

In the foregoing table it is interesting to note that carbohydrates alone are not sufficient. Pure starch is of no use as a food to these larvae: boiled starch would appear to have a slight value, but under the precise conditions it is questionable whether what little food value it has is not in reality due to a subsequent bacterial conversion of the boiled starch and to traces of nitrogenous matter with which the commercial product is associated.

However, when we get to foodstuffs containing proteins the whole story is changed and the development of the larvae exhibits a very different picture. It is thus demonstrated (though not for the first time) that the larvae of *Aëdes argenteus* can flourish on many artificial foodstuffs, many of which, perhaps supreme among them being bread, support the development of larvae infinitely better than the food supplies of the average breeding-place. I have in fact now reared on a bread diet at my laboratory at Wisley specimens of *Aëdes argenteus* whose size, decorative magnificence and rate of growth rival and even surpass these characteristics of the finest specimens bred in the tropics. Apart, however, from the great value of bread, etc. as a larval diet, a most interesting and significant matter is brought to light in that none of the larvae reared in bread cultures have ever shown what has been termed the phenomenon of "suspended development" which is so frequently exhibited among larvae in a "natural" environment.

8. Observations on the influence of the artificial foods used in Table I.

Previous work had indicated that for some reason the development of the *Aëdes argenteus* larvae was adversely affected by acid media, and as it was found, moreover, that the *p*H index in the artificial food tubes varied considerably from day to day, the index of each tube was kept at about *p*H 8 by the addition of dilute ammonia in distilled water when necessary to maintain that index in order to preserve the degree of alkalinity most favourable to the larvae. Other experiments in which the *p*H in paired tubes was controlled were later undertaken, using natural and artificial foodstuffs, in acid and alkaline water, the results of these experiments being given in Table II.

9. Experiments on the influence of the hydrogen-ion concentration (H-i C.) in different media.

In most instances, if the *p*H of the media indicated acid conditions, the development of the larvae was either seriously affected or the larvae soon died. However, in these experiments, two observations of considerable interest and importance were established. It will be noticed that in each case one of the paired tubes was made acid and the other alkaline, to the extent of *p*H 3 and

Table II.

(Each tube contained 50 c.c. distilled water, and 6 1st instar larvae.)

Tubes	*Medium*	*H-i C.*	*Remarks after an interval of 3 days*
A 1–A 7	Distilled water	*p*H 3 (acetic acid)	*All larvae dead.* *p*H 4
A 8–A 14	„	*p*H 10 (ammonia)	3–6 larvae alive in each tube, but all quite small. *p*H 9
B 1–B 7	0·5 % bread	*p*H 3 (acetic acid)	*All larvae dead.* *p*H 4
B 8–B 14	„	*p*H 10 (ammonia)	5–6 larvae alive. Growth vigorous. *p*H now 8
C 1–C 7	0·1 % bacto-peptone	*p*H 3 (acetic acid)	*All larvae dead.* *p*H 4
C 8–C 14	„	*p*H 10 (ammonia)	3–5 larvae in each tube. Some large, others small. *p*H now 8
D 1–D 7	Grass infusion	*p*H 3 (acetic acid)	*All larvae dead.* *p*H 4
D 8–D 14	„	*p*H 10 (ammonia)	All larvae alive, but small. *p*H 6·5
E 1–E 7	Distilled water	*p*H 3 (hydrochloric acid)	*All larvae dead.* *p*H 3
E 8–E 14	„	*p*H 10 (ammonia)	3–6 larvae in each tube, but no perceptible growth. *p*H 9
F 1–F 7	0·5 % bread	*p*H 3 (hydrochloric acid)	*All larvae dead.* *p*H 4
F 8–F 14	„	*p*H 10 (ammonia)	4–6 larvae alive in each tube. Growth vigorous. *p*H 8
G 1–G 7	0·1 % bacto-peptone	*p*H 3 (hydrochloric acid)	*All larvae dead.* *p*H 3
G 8–G 14	„	*p*H 10 (ammonia)	All larvae alive, growth moderate. *p*H 8·5
H 1–H 7	Distilled water	*p*H 3 (sulphuric acid)	*All larvae dead.* *p*H 3–4
H 8–H 14	„	*p*H 10 (ammonia)	*All larvae dead.* *p*H 10–*p*H 9
I 1–I 7	0·5 % bread	*p*H 3 (sulphuric acid)	*All larvae dead.* *p*H 3–4
I 8–I 14	„	*p*H 10 (ammonia)	All larvae alive. *p*H 8. Growth rapid
J 1–J 7	0·1 % bacto-peptone	*p*H 3 (sulphuric acid)	4 larvae survived: two pupated eight days later. *p*H 8
J 8–J 14	„	*p*H 10 (ammonia)	4–6 larvae survived in each tube. *p*H 8·5. Large specimens
K 1–K 7	Grass infusion	*p*H 3 (sulphuric acid)	*All larvae dead.* *p*H 5
K 8–K 14	„	*p*H 10 (ammonia)	All larvae alive. Fair-sized specimens. *p*H 8
L 1–L 7	Distilled water	*p*H 3 (citric acid)	4–6 larvae still alive. *p*H 3
L 8–L 14	„	*p*H 10 (ammonia)	All alive; small. *p*H 8
M 1–M 7	0·5 % bread	*p*H 3 (citric acid)	All alive; small. *p*H 6·5
M 8–M 14	„	*p*H 10 (ammonia)	All alive; large. *p*H 7·5
N 1–N 7	0·1 % bacto-peptone	*p*H 3 (citric acid)	All alive; large. *p*H 8
N 8–N 14	„	*p*H 10 (ammonia)	All alive; large. *p*H 8
O 1–O 7	Grass infusion	*p*H 3 (citric acid)	3 larvae alive, but still quite small. *p*H 6·5
O 8–O 14	„	*p*H 10 (ammonia)	All alive; fair size. *p*H 8
P 1–P 7	Distilled water	*p*H 3 (phosphoric acid)	4 larvae alive; quite small. *p*H 3
P 8–P 14	„	*p*H 10 (ammonia)	All alive; quite small. *p*H 8·5
Q 1–Q 7	0·5 % bread	*p*H 3 (phosphoric acid)	*All dead.* *p*H 3–4
Q 8–Q 14	„	*p*H 10 (ammonia)	All larvae alive; large. *p*H 8
R 1–R 7	0·1 % bacto-peptone	*p*H 3 (phosphoric acid)	*All larvae dead.* *p*H 3–4
R 8–R 14	„	*p*H 10 (ammonia)	All larvae alive; fair size. *p*H 8·5
S 1–S 7	Grass infusion	*p*H 3 (phosphoric acid)	1 larva alive, small. *p*H 3
S 8–S 14	„	*p*H 10 (ammonia)	All alive; fair size. *p*H 8·5
T 1–T 7	Distilled water	*p*H 3 (nitric acid)	*All larvae dead.* *p*H 3
T 8–T 14	„	*p*H 10 (ammonia)	3–6 larvae alive in each tube, but no perceptible growth
U 1–U 7	0·5 % bread	*p*H 3 (nitric acid)	*All larvae dead.* *p*H 4
U 8–U 14	„	*p*H 10 (ammonia)	4–6 larvae alive in each tube. Growth vigorous. *p*H 8
V 1–V 7	0·1 % bacto-peptone	*p*H 3 (nitric acid)	2–3 larvae in each tube alive. *p*H 6·5
V 8–V 14	„	*p*H 10 (ammonia)	All larvae alive. Growth moderate. *p*H 8·5

*p*H 10 respectively, by the addition of dilute acids and ammonia. These tubes, containing in each 6 1st instar larvae from eggs which had been laid on freshly distilled water (as far as possible to prevent gross bacterial contamination) were then placed in an incubator lit by diffused daylight and in darkness during the night, at a temperature of 30° C., where they were allowed to make what growth they could during a period of 3 days without further control. In those cases where dilute sulphuric, hydrochloric, nitric and acetic acids had been used to render one of the tubes of each pair acid, and the index during the interval of 3 days had remained below *p*H 6·5 all the larvae invariably died; whereas, if during the 3-day period the index had changed to *p*H 6·5 or above, the larvae survived. For a time this was considered as possible evidence that the acidity or alkalinity of the medium had in itself a direct influence upon the larvae. But in the experiments in which citric and phosphoric acids had been employed the larvae in distilled water were found to tolerate acidity, and the evidence furnished by the use of the other acids thus became untrustworthy.

In subsequent experiments using bread in distilled water as the larval food supply, I was puzzled to account for the fact that while in all these rearing-jars the larvae did extremely well for some days, nevertheless, the larvae in some of the jars would then often die rapidly while the other larvae in companion jars completed their growth and metamorphosis perfectly, and at an excellent rate. Tests made of the *p*H of the media in both sets of jars disclosed the fact that the reaction in the former cases was acid, and in the latter cases alkaline.

In spite of occasionally conflicting observations it was patently indisputable that acidity, in the majority of instances, was in some manner correlated with unfavourable conditions to the larvae. If a small quantity of bread (about 5 grm.) is added to about 500 c.c. of distilled water in an open glass jar, the index of the water after a lapse of some hours at 30° C. will generally be found to be about *p*H 8, but if the water in the jar be tested again after an interval of from 2 to 4 days the index will often have changed in the interval to about *p*H 4 or less, caused by the bacterial or yeast fermentation of the bread. We may suggest that what causes the death of the larvae under these conditions is not the acid reaction of the water but the production of a high concentration of fermentation or bacterial toxins. That the bacterial flora varies to a remarkable degree according to the reaction of the media is a well-known fact which was again confirmed in these experiments by Major H. C. Brown, C.I.E., of the Wellcome Bureau of Scientific Research, to whom I submitted for bacterial examination samples of water taken from both the acid and alkaline jars. Major Brown informed me that in the acid water a mixed culture of non-motile gram-negative organisms was present, while the alkaline water held an almost pure culture of actively motile organisms, and from this it was inferred that pathogenic organisms and their toxins peculiar to the acid water might well account for the death of the larvae in these jars. However, it happens that if an acid bread jar is permitted to remain in the incubator for a further few days

after the death of the first larvae it contained, other larvae then may frequently be added to the water of the jar and they will often develop at a high rate perfectly. *If under these circumstances the reaction of the water is again tested its reversion to alkalinity is disclosed.* Consequently, as I have said, a certain amount of confirmation was given by these observations to the belief that the reaction, *per se*, either directly or indirectly, might be the principal factor in favouring or impeding the larval development.

Nevertheless, I was faced with one other important factor which was unknown and uncontrolled, namely, the precise influence the bacterial cycle had indirectly on the larvae by conversion of the food materials, and directly by toxic and pathogenic action. It was therefore realised that further progress in the effort to determine whether the reaction of the medium itself directly influenced the development of the larvae necessitated the elimination of bacterial interactions.

10. Experiments on the influence of changes in the *p*H index under sterile conditions.

As no conclusive evidence as to what part in larval development the *p*H index played could be obtained by the experiments already undertaken, it was decided to repeat the work if possible under sterile conditions. The work of Bacot (1917), Guyénot (1917) and Barber (1927) in the production of sterile cultures of larvae led me to attempt to adopt their technique for the production of a large supply of sterile cultures of *Aëdes argenteus* larvae. Although Bacot succeeded in obtaining a few sterile cultures of larvae by disinfecting the exterior surfaces of the eggs with dilute lysol he had on the whole little success. On the other hand, Barber had much better results by treating the ova with 80 per cent. ethyl alcohol while they were stranded on cotton-wool held in a perforated spoon, dripping the alcohol from 5 to 10 minutes over the eggs. Both Bacot's and Barber's methods were tried. Bacot's method gave very inconsistent results, and killed too high a percentage of the ova to make the method of any practical value in experiments where large numbers of clean larvae were required. Barber's method was found to be satisfactory, but caused much waste of time in the sterilisation treatment, and the percentage of sterile cultures obtained was rather low.

A technique had therefore to be evolved which would be more satisfactory by enabling a high proportion of sterile cultures to be obtained, and one which would permit large numbers of eggs to be treated successfully at one time.

After a number of different means had been employed, the following technique was developed, and provides a method which is both very simple and which gives large numbers of bacteriologically sterile ova.

11. A METHOD OF OBTAINING BACTERIOLOGICALLY STERILE CULTURES OF *AËDES ARGENTEUS*.

Efforts were at first made to disinfect the exterior surfaces of the eggs by removing the ova from the water on which they had been laid, in the following manner. A piece of white paper about $\frac{1}{2}$ in. square was placed in a small watch-glass. The watch-glass was then half-filled with 80 per cent. ethyl alcohol, and the eggs, lifted from the water with the aid of a camel-hair brush, were placed on the surface of the alcohol immediately above the small piece of submerged paper and, by being themselves submerged, sank through the alcohol on to the surface of the paper, where they could be kept under observation. The eggs were kept in motion on the paper by means of a blunt needle manipulated under a binocular microscope for from 5 to 10 minutes, and later, with extreme care, the alcohol was pipetted out of the watch-glass, and the paper with the eggs adhering to it was lifted and dropped by sterile forceps into the autoclaved bread-culture tubes. On the whole the results from this technique were disappointing. It was extremely difficult to keep the eggs on the paper as the alcohol was drawn off, the percentage of sterile cultures was low (spore-bearing bacteria contaminating the tubes), and to our surprise in a large proportion of the tubes a comparatively rare and unidentified species of fungus developed. The origin of this fungus was finally traced to spores on the paper we had used, although it was at first assumed that the alcohol treatment would effectually sterilise the new and apparently clean paper as well as the ova.

After several attempts to devise a better means of controlling the ova in the watch-glass, it was found that ordinary circular No. 2, $\frac{1}{2}$ in. diameter glass cover-slips could be rapidly converted into small glass "boats" having upward and inward turned edges which gave us the means of handling the ova perfectly. If a No. 2, $\frac{1}{2}$ in. diameter cover-slip is held by one edge in an old pair of forceps and is brought into a gas flame from a Bunsen burner in a horizontal position, it will be found that the flame softens the glass rapidly and that the opposite edge of the cover-slip to that held by the forceps bends over and inwards towards the centre. When this has taken place, by removing the cover-slip from the flame and allowing it to cool, the process may be repeated in the case of the other edge, and we then have a small glass "boat" or "shell" similar to the illustration, Fig. 1. The value of these "boats" is that the form of their upturned edges ensures the absolute security of the ova in all disinfectant

Fig. 1. Glass "boat" used for sterilising the ova of *Aëdes argenteus*.

treatment, and being of glass they may be "flamed" before use. A number of these boats should be made at one time to ensure the requisite supply.

The "boats" are used in place of the paper squares, and if the ova to be disinfected are placed on the surface of the alcohol in the watch-glass immediately over one of these submerged "boats" the eggs can be made to sink through the alcohol into the boats. Rocking the boat by pressure with the point of a needle will cause the ova to be kept in motion, or they may be directly moved about by the needle-point, to assist the action of the disinfectants. In any case they are then perfectly under control and run no risk of being lost even when transference is effected to the culture tubes.

Although by the use of these "boats" the proportion of our sterile cultures of larvae was markedly increased we still encountered a large number of failures due to contamination by spore-bearing organisms which had resisted the action of the disinfectants. Various disinfectants were tried including 5 per cent. aqueous carbolic acid, 5 per cent. lysol, 1/500–1/1000 brilliant green, 10 per cent. eusol, alcoholic mercury chloride, and hydrogen peroxide, but while the protection afforded to the mature embryo by the egg-shell was little short of astonishing, effective bacteriological sterilisation of the eggs by these reagents too often resulted in injury or death to the embryos also.

Excellent results, however, were finally obtained by the use of 5 per cent. potassium soap solution in distilled water as a preliminary treatment, followed by immersion in 80 per cent. ethyl alcohol. Apparently one action of the soap solution is either the partial or complete solution of the enveloping membrane, and by lowering the surface tension, the insurance that in the subsequent alcohol treatment the alcohol establishes complete contact with all the microscopical irregularities of the egg on which bacteria lodge. Incidentally, after the use of the soap, no trouble is experienced with adherent air bubbles, which otherwise are often difficult to detach from the ova. The soap solution may also have some preliminary direct detrimental effect upon the bacteria themselves, but whatever the sum of the actual effects of the soap, its use seems greatly to favour the bactericidal action of the alcohol.

In order to ensure success as far as possible the fed female mosquitoes from which the ova were to be obtained were kept in clean glass jars for 36 hours at 30° C. after feeding, and at the end of this period the insects were transferred to other glass jars containing sterilised distilled water on which the mosquitoes could oviposit. The jars were plugged with sterile cotton-wool. No difficulty was experienced in getting the insects to lay on this perfectly clean water, proving that the control of their impulse not to oviposit in water containing no larval food must be limited or non-existent. The eggs obtained in this manner were allowed to incubate for from 66 to 72 hours before being treated with disinfectants as not before this period are the embryos able to withstand desiccation (MacGregor, 1916) and it was concluded that the embryos were consequently more secure from adverse influences as a whole.

These eggs were lifted by means of sterile platinum loops or by sterile

"spoons" constructed from sterile capillary glass tubes, and placed first on to the surface of a 5 per cent. potassium soap solution in a watch-glass wherein one of the glass "boats" was already immersed. After the eggs had been submerged in the soap solution and had fallen into the glass "boat" they were kept in constant gentle motion for a period of from 5 to 7 minutes, and with the aid of a pair of sterile forceps the "boat" containing the eggs was carefully transferred to another watch-glass containing 80 per cent. ethyl alcohol, after draining the "boat," by tilting, of as much of the soap solution as possible. The same procedure in regard to keeping the eggs in gentle motion was followed while they remained in the alcohol solution, and in this solution it was found quite safe and advisable to keep them for from 10 to 15 minutes. At the end of the period the "boat" was lifted with sterile forceps out of the alcohol, partially drained of the fluid by tilting, and then dropped into the culture tube under the usual aseptic precautions.

Often even after the 15–20 minutes' immersion which they had had in the disinfectant solutions the eggs would hatch almost as soon as the "boat" reached the bottom of the tube of culture medium. Out of hundreds of experiments of this nature it can be affirmed that in no single instance has failure to hatch occurred, although the incubation period has occasionally, for some reason, extended for as long as 48 hours after the eggs were placed in the culture tubes. Usually, however, if the eggs are from 66 to 72 hours old at the time of disinfection and receive the treatment outlined above they will hatch either immediately or within a few minutes or within an hour or two after entering the culture tubes, when kept at 30° C. There is on the whole undoubtedly a stimulus to hatching given by this treatment, as we have proved by control observations. The percentage of bacteriologically sterile cultures obtained by this technique is high, provided that every step in the process is guarded by aseptic precautions. On an average we now get about 82 per cent. of our cultures sterile. Thus ostensibly the soap-alcohol treatment is quite capable of killing the comparative paucity of bacteria borne by eggs which are obtained in the manner described. Our non-sterile cultures are most frequently due to contamination with spore-bearing organisms, and the origin of these organisms seems to be principally the dejecta of the parent, since if the mosquitoes are not transferred before actual oviposition from the containers they have inhabited during the digestion of their blood meal to clean containers wherein to oviposit, the percentage of non-sterile eggs and cultures is greatly increased.

12. The sterile cultures of *Aëdes argenteus*.

In all my work on the value of different artificial foodstuffs to mosquito larvae, nothing has been found to suit the larvae of *Aëdes argenteus* as well as bread. A culture medium composed of distilled water, 50 c.c., and "standard" bread, 0·5 grm., contained in glass boiling-tubes and autoclaved at 120° C. for from 15 to 20 minutes, to which 10–20 sterile ova are added has therefore

become the standard procedure in the investigations now to be described. In this medium and under these conditions at an incubator temperature of 30° C. the development of the larvae is successful, rapid, and generally at a uniform rate of from 9 to 12 days from the hatching of the eggs to pupation, and from 1 to 3 days longer for the emergence of the adults. The specimens are of good average size, sexually potent (unlike the case of Guyénot's sterile cultures of *Drosophila*) (Guyénot, 1917), and with a strain under sterile conditions metamorphosis has been maintained for four generations with no apparent decline in vitality. Nevertheless, it must be admitted that strains raised under sterile conditions rarely seem to reach a magnificence in general bodily development comparable with that of insects which are raised on a bacteria-contaminated alkaline bread medium.

The true significance of this is difficult to compute or to demonstrate. Possibly the bacteria themselves furnish the larvae with some important accessory food factor (vitamins ?) from their own bodies or produce this factor by conversion of the materials of the bread. Or it may be that the bacteria have nothing whatever to do with the matter, and that it is simply due to the fact that in these cultures the bread has not been treated in the autoclave and a proportion of the yeast-vitamin has survived the bread-baking process. Yet it is questionable whether vitamins can affect the life of some insects at least, since many exhibit a successful existence on such materials as paper, starch-paste, spun cotton and silk, silicate of cotton, etc.

An effort was made to determine whether the successful development of the larvae in the autoclaved tubes was associated with the presence of vitamins which had so far resisted destruction by the heat-pressure bacterial sterilisation. A batch of culture tubes was therefore given 2 hours in the autoclave at 130° C., representing a pressure of approximately 39 lb. per square inch. When removed from the autoclave the colour of the fluid in the media tubes was observed to have changed from neutrality to a decided brown, and batches of ova placed in these tubes hatched, but died soon afterwards, while larvae raised under aseptic conditions when transferred to these tubes died within 12–48 hours. The idea of accounting for this on the supposition of destroyed vitamins thus looked promising until 10 per cent. of this medium was added to the medium in tubes wherein the larvae were doing well. The result was the prompt death of the larvae, and the only conclusion possible was that their death was due to the creation of toxic substances by partial heat decomposition of the bread, and that it had no connection with either the presence or absence of vitamins.

13. Experiments on the effect of altering the *p*H of the sterile bread cultures.

Once sterile cultures wherein larvae successfully developed were available we finally had all the essential factors under control, *i.e.* light, temperature, organic materials in the larval environment, and the food supply. Alterations of the *p*H index, if it had any effect, in all probability could now have only a

direct effect upon the larvae themselves. As a 0·01 *N* solution of hydrochloric acid is dissociated to the extent of 96·4 per cent. (*p*H 2·02) the concentration of H-ions for a given *p*H is brought about by the minimum necessary addition of acid, I decided, therefore, to employ hydrochloric acid for the first tests. A 0·1 *N* solution of HCl in distilled water was made up, sealed in glass ampules, and sterilised at 125° C. for 20 minutes. By tests on a few bread culture tubes the necessary number of drops from a sterilised capillary pipette of this acid solution which had to be added to the tubes to give a particular *p*H index was determined—using the B.D.H. Universal Indicator.

A series of tubes containing standard bread culture media and 10 larvae in each were given a reversal of index from the normal (*p*H 8) to *p*H 6 and *p*H 3.

The immediate effect of this under non-sterile conditions would have been to affect adversely the larval development, but now under sterile conditions nothing of the kind was found to occur, and the larvae in the aseptic tubes seemed to develop quite as well at *p*H 4 or *p*H 6 as did others at *p*H 8. Had the larvae under these conditions showed signs of being adversely affected it would have been open to question whether the Cl anions were not responsible. However, that was not the case. The experiments, nevertheless, were repeated using dilute sulphuric, nitric, phosphoric, acetic and citric acids and the observations were in all cases confirmed.

14. The development of the larvae of *Aëdes argenteus* in a strongly acid medium.

In view of the fact that we had obtained successful development of the larvae of *Aëdes argenteus* in acid media under aseptic conditions and that we could not do so in non-sterile bread and other media, a further search was made to obtain if possible some artificial food supply which the larvae could utilise, and which would not require subsequent bacterial intervention to produce a condition of acidity. Such a medium was finally discovered in dried raisins and other dried fruit. If half a dried raisin is placed in 50 c.c. of distilled water and allowed to stand for from 5 to 15 minutes, and the medium is then shaken up, it indicates a *p*H of from 4 to 6·5. Clean, but not bacteriologically sterilised ova placed in this medium hatch normally, and develop most satisfactorily although the *p*H index will be found to show rapidly increasing degrees of acidity. At the end of 24 hours the medium will often show a *p*H of 2–3, though nevertheless the larvae are unaffected, and in this medium develop almost as well as they do in the standard bread medium. Obviously, it was now most interesting to know what would be the result of altering the normally acid reaction of this medium to a condition of alkalinity, and by this means to obtain the converse expression of our earlier experiments.

Five out of a group of ten tubes containing in each half a dried raisin in distilled water and 10 larvae of *Aëdes argenteus* had the reaction of the water made alkaline (*p*H 8) by the addition of dilute ammonia. The other five tubes registered a *p*H of 4. The ten tubes were placed in an incubator at 30° C. and

24 hours later the first set of five tubes registered *p*H 5, and the second set *p*H 2–3. By this time the larvae in both sets of tubes were growing well, and a further addition of dilute ammonia was added to the first set sufficient to give a reading of *p*H 9. As the tendency in this set of tubes was to become acid in spite of the additions of ammonia, one more addition of ammonia was made at the end of the next 12 hours. The index was then stable at *p*H 8·5–9, and remained so until the end of the experiment. During the next 12 hours a sudden change in the condition of the alkaline tubes set in; the fluid rapidly became opaque with a dense growth of micro-organisms, considerable amounts of CO_2 were evolved, and before the next 12 hours (48 hours in all) had passed, all the larvae were dead. In the acid set of tubes the fluid was still clear and remained so until the larvae developed to adults 14–16 days later.

Following the procedure of the earlier experiments, the dead larvae in the alkaline tubes were removed, and the medium was made to revert to acidity (*p*H 4) by the addition of dilute acetic acid, and it was then found that other larvae placed in the formerly fatal medium would develop most satisfactorily.

Thus strong confirmation is given to the belief that in many instances the *p*H index furnishes an index also as to the adverse or favourable association of chemical and biological factors upon which the development of particular species depend.

15. The optimum concentration of the food supply.

While mosquito larvae develop best in waters rich in food materials our experiments have shown conclusively that an over-abundance of food is prejudicial to their health and often fatal. Using bread as the larval diet it is found that the optimum concentration is represented by the addition of 0·1 per cent. of bread by weight compared with the weight of the water in the container when there are approximately 100–200 larvae in approximately 250 c.c. of distilled or tap water. Under these conditions this amount of bread at a water temperature of 30° C. results in the development of a slight turbidity of the water within from 24 to 48 hours. As is well known, the feeding action of the larvae in turbid water often brings about the "clearing" of such water so that it again resumes its transparency, and it is the exhibition of this phenomenon, and its maintenance, that should be our aim in the establishment of what represents the optimum concentration of the food supply. If shortly after the first 48 hours the turbidity of the water begins to disappear, small additions of bread may be made to the water in the containers every 48 hours, or at any time before or after this period if transparency reappears.

A point of great interest is the fact that the larvae will tolerate a high degree of turbidity in the water due to the addition of an excessive amount of bread as long as the reaction of the water is alkaline, but, just as soon as any marked degree of acidity develops from bacterial fermentation of the culture medium, the larvae are in danger, and frequently die rapidly. The preceding accounts of our experiments on the significance of the *p*H index, and the development

of larvae under sterile conditions, make it clear that the adverse influence upon larvae in the presence of any excess of food in such cases is due either to the production by inimical bacteria of substances toxic to the larvae or to the development of pathogenic bacteria within or on the bodies of the larvae themselves. It should be noted that in the sterile culture tubes, what would have been an excess of bread otherwise has no adverse influence upon the larvae within these tubes.

The same thing is true in regard to all the other artificial foods used in our experiments, and no doubt is equally true in regard to the food supply under natural conditions.

16. The "suspended development phase."

In the light of the results of our work it now seems certain that the explanation of the "suspended development phase" frequently exhibited by the larvae of many species, where at first some of a batch of larvae do well and complete their metamorphosis, while others of the same batch show an increasingly slow rate of development, is mainly due to a progressively increasing food shortage. The basis of the food supply may often be present in ample amount, but the actual food materials are unavailable to the larvae in consequence of the absence of the required micro-organisms for its proper conversion to a nutritive diet, which in turn is frequently due to an alteration of the *p*H of the water making the medium no longer fit for the particular micro-organisms which are normally co-existent. The fact that it is commonly possible by suitably altering the *p*H index either to acidity or to alkalinity as the particular case demands to end the "suspended development phase" and to reinstate the normal development, lends considerable confirmation to this belief. The phenomenon of "suspended development" is most often encountered with larvae living in water containing dried leaves and twigs such as in the normal habitat of the tree-hole breeding species. "Suspended development" has not, in my experience, been met with in larvae situated in their natural habitat, and it is only when they are brought to the laboratory that this phenomenon manifests itself. It is just possible that the "suspended development phase" is an inherent characteristic of certain species of larvae and that it is correlated with "overwintering" as larvae. In nature the lowering of the atmospheric temperature may bring about the temporary disappearance of certain micro-organisms upon which the production of the larval food supply is dependent, and thus the same effect may be established as we get under laboratory conditions where the effect is brought about by agencies other than changes of temperature.

17. Summary.

1. Previous work by the writer drew attention to the fact that the *p*H index of natural waters was in some manner correlated with the presence or absence of particular mosquito larvae.

2. The observation led other investigators to study this hypothesis, and a short summary of their published records is given.

3. The actual significance of the *p*H index was, however, obscure, and the present work has been undertaken to attempt to demonstrate the actual significance of the *p*H in mosquito breeding-places.

4. By gradually eliminating first one and then another of the interacting factors, the writer has been able to demonstrate (*a*) that if the *p*H of the normal environment is changed the development of the larvae is adversely affected; (*b*) that under bacteriologically sterile conditions the foregoing statement is no longer true; and (*c*) that consequently the acid or alkaline reaction of the medium, within ordinary limits, has no direct effect upon the development of the larvae.

5. Nevertheless, it is shown that the influence of the *p*H is of unquestionable importance and that its significance lies in the fact that, under natural conditions, it indicates the favourable or unfavourable association of chemical and biological factors in the breeding-places upon which the successful or unsuccessful development of the larvae depends.

6. It may therefore be stated that although the *p*H index is not an infallible guide, it does in the majority of instances provide a reliable index as to whether the necessary combination of chemical and biological factors in any given water are such that they will permit the development of the larvae of particular species. Some species find their optimum conditions for development in waters which are acid, others in waters which are alkaline, and it can be shown that to alter the *p*H of such waters is often to bring about a complete change in the biological group associations, which frequently means the acquired abnormal dominance of factors unfavourable to a particular species of larvae. Clearly, the system is to some extent elastic, and in rare instances what are in general acidophile larvae may be found in waters showing alkalinity.

7. The chief conclusion is, however, that in most instances in various localities the larvae of certain species do actually show a restriction to waters exhibiting a *p*H index within a definite short range, and that the *p*H index is consequently often a reliable index also as to whether the chemical and biological group associations will favour or preclude the successful development of such larvae.

8. A new technique is described for the production of a high percentage of successful cultures of *Aëdes argenteus* larvae and pupae under bacteriologically sterile conditions.

9. The phase of "suspended development" has been investigated and is found to have its probable explanation in the temporary or complete disappearance of micro-organisms on which the conversion of the organic materials of the environment, to a suitable larval diet, depends.

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A NEWLY OBSERVED REACTION OF CERTAIN SPECIES OF MOSQUITOES TO THE BITES OF LARVAL HYDRACHNIDS.

PRELIMINARY CONTRIBUTION.

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(With 3 Text-figures.)

In the course of a Mosquito Survey carried out in the neighbourhood of Birmingham on July 31 and August 1, 1928, a large number of female mosquitoes of the two species *Aëdes* (*Aëdes*) *cinereus* and *Aëdes* (*Ochlerotatus*) *annulipes* were collected and subsequently examined.

It was found that one of the *A. annulipes* and the majority of the *A. cinereus* adults were infested with larval mites resembling those which, some years previously, had been found attached to female adults of *Anopheles maculipennis* and *Aëdes maculatus* collected at Hayling Island.

Further examination of the infested adults disclosed the presence of dark, serpentiform, tubular processes, originating at the points where the mouth-parts of the larval mites were attached to the host, and penetrating within the abdomen to distances varying from 0·5 to 1·0 mm. A number of similar "tubes" unaccompanied by mites were also present, these having been caused presumably by mites subsequently detached during collecting or transport. The abdomen of one of the infested adults is shown in Fig. 1.

An enlarged illustration of one of the larval mites with its "tube" is shown in Fig. 2, and of a tube alone in Fig. 3[1].

Most of these tubes have their origin on the dorsal side of the abdomen, although a considerable number (both with and without attached mites) originate on the ventral side. The total number of tubes discovered during the examination of 17 adults amounts to 69, 54 of which occur in the dorsal area. The greatest number of tubes present in any one specimen is 12, 11 of these originating in dorsal segments. In all but three cases, the points of origin of the tubes are situated in juxtaposition to the proximal or distal margins of the segments; the frequency with which the different segments are affected being indicated in Table I.

For the purposes of this table, the dorsal and ventral surfaces of the abdomen are assumed to be divided longitudinally into three equal parts;

[1] As previously mentioned, some adults of *Anopheles maculipennis* ♀ infested with larval mites (unidentified) were collected at Hayling Island in 1924. An examination of some microscope slides prepared at that time has disclosed, in the abdomen of one of the said specimens, a "tube" of obviously similar nature.

the number of tubes occurring (*a*) in the "middle third," and (*b*) in the two "outer thirds" combined, being given in separate columns.

With the exception of the 8 cases referred to at the foot of the table, all the tubes enumerated have their origin on or near the *proximal* margins of the segments.

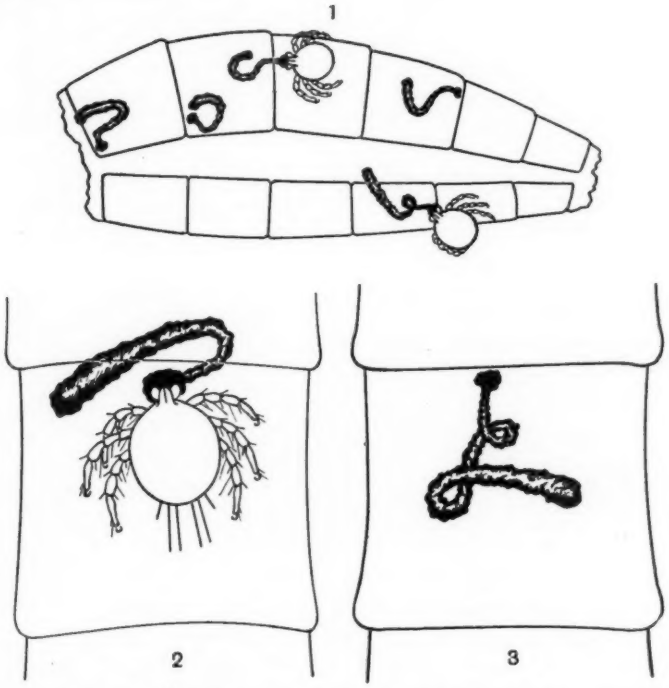

Fig. 1. Lateral view of abdominal segments II–VII of *Aëdes cinereus* ♀, showing attached larval mites and "tubes." (×14.)

Fig. 2. Dorsal abdominal segment of *Aëdes cinereus* ♀, with larval mite and "tube." (×33.)

Fig. 3. Dorsal abdominal segment of *Aëdes cinereus* ♀, showing "tube" caused by larval mite. (×33.)

In all the cases in which the distal margins of the segments are attacked, the mouth parts of the mites are attached at points lying on the junction of the segment with the flexible intersegmental membrane.

As will be noted from the illustrations, the point of origin of a tube is, in most cases, surrounded by a dark mass. These dark masses, which are apparently of similar composition to the tubes themselves, are specially prominent in the cases where they occur in the distal margins.

As the illustrations show, the tubes increase in diameter in proportion to their distance from the point of origin. They are irregularly pitted and wrinkled, though possibly these irregularities may be wholly or largely due to drying.

Table I. *Segmental distribution of* 69 *"larval mite tubes" found in the abdomens of* 17 *female mosquitoes* (Aëdes cinereus).

Abdominal segment number	Tubes originating on dorsal surface			Tubes originating on ventral surface		
	(*a*) Middle third	(*b*) Outer thirds	Segment total	(*a*) Middle third	(*b*) Outer thirds	Segment total
I	0	0	0	0	0	0
II	2	5	7	0	1	1
III	8 (*a*)	7	15	1	0	1
IV	5	6	11	3	1 (*b*)	4
V	10 (*d*)	5	15	4 (*e*)	1	5
VI	3 (*f*)	3	6	1	1	2
VII	0	0	0	2 (*c*)	0	2
		Total (Dorsal)	54		Total (Ventral)	15

NOTES: (*a*) Three tubes originating on distal margin of segment. (*b*), (*c*) One tube originating on distal margin of segment. (*d*), (*e*), (*f*) One tube originating midway between proximal margin and centre of segment.

It appears probable that the tubes do not penetrate deeply into the abdomen, but remain near the surface.

The tubes show no trace of any helical structure, such as is found in the tracheal passages.

In the case of abdomens prepared for examination by immersion in a cold solution (5 per cent.) of caustic potash, the tubes retain their dark colour; but this disappears if the specimen is boiled for half an hour in a similar solution. Staining tests, carried out with both safranin and picric acid, show that the tubes are composed of chitinous material.

Specimens of the mites have been submitted to Mr Cecil Warburton, M.A., of Cambridge University, who states that they are certainly larval Hydrachnids, and that Nalepa in the "Tierreich" has figured a very similar form as the larva of *Lebertia tauinsignata* (Fam. Hydrachnidae).

As far as can at present be determined, these tubes are the product of a protective reaction occurring in the mosquito to oppose some kind of infective invasion originating in the bite of the parasite. No such processes, however, appear to have been hitherto described, though attention may be directed to a paper relating to the Harvest Bug (*Leptus autumnalis*) published by Brandis in 1897[1]. One of Brandis' figures, which illustrates a section of the skin of a hedgehog attacked by *Leptus*, shows tubular processes issuing from the mouth-parts of the mites and penetrating deeply into the host. Apparently Brandis regarded these "funnel-shaped" (*trichterförmig*) processes as being mouth-parts of the parasites; but it seems possible that he had actually observed (and misunderstood) a reaction of similar character to that herein described.

[1] Brandis, F. (1897). Ueber *Leptus autumnalis*. *Festschr. d. Provinzial Irrenanstalt*, Nietleben.

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ON A NEW LOUSE FROM A CEYLONESE SHREW.

By JAMES WATERSTON, D.Sc.

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Ancistroplax n.g.

Anoplura without eyes; with four segmented antennae not dimorphic in the sexes; 1 well developed sensorium on 4th antennal joint postero-basally; head considerably contracted posteriorly. Segments I–III closely united, more distinctly separated from the rest of the abdomen than the succeeding segments are *inter se*. 2nd sternite divided. 3rd cap-shaped.

Pleurites on segments IV–VI divided; the others membranous or lightly chitinised along the mid line, the thin area widening round the spiracles so that the latter are conspicuous. These sclerites are of the *Hoplopleura* type, and strongly developed. The two bristles medianly approximated.

♀. Fore and mid legs not disproportionate; the latter only a little longer. Tergites IV–VII and sternites IV–VII with three rows of stout flattened bristles.

♂. Fore legs distinctly smaller than mid legs. Tergites IV–VII with two rows of stout bristles, tergite VI modified (Fig. 1 *c*) with one irregular row (2 coalesced), sternites IV–VI two rows. Statumen penis well developed.

Genotype the species described below.

Ancistroplax n.g. appears to be closely related to *Schizophthirus* and *Euhaematopinus*. The three genera agree in having the 2nd sternite divided, and in the facies of the pleurites. As regards the antennae *Ancistroplax* is intermediate, as four joints are clearly indicated.

From *Schizophthirus* it differs in the antennae and chaetotaxy, and from *Euhaematopinus* in the chaetotaxy and simple legs, while its male is quite unique. The striking features of this sex in the genotype, however, may be specific.

Ancistroplax crocidurae n.sp.

♀. Total length 1·25. Breadth (segment V) about 0·37. Head 1 = 0·16. Breadth 0·12. Antennae 1 = 0·1. Thorax, breadth 0·16. Thoracic spiracles 0·02. Abdominal spiracles 0·018.

Gonopods with 3 decussating bristles and 3–4 more between these sclerites and side.

Shape of pleurites (Fig. 1 *a*, *b*).

The first is small and weakly chitinised.

ABDOMINAL CHAETOTAXY.

Segment	Tergite	Pleurite	Sternite
I	1, 1	0	0
II	1, 1, 2, 1, 1	2 (short, lateral) 1, 1	1, 1
III	2, 2	2 (long, median) 1, 1	3, 3
IV–VI	2, 2	2 (minute, median) 1, 1	2, 2
	2, 1, 2		2, 1, 2
	3, 3		3, 3
VII	2, 2	2 (long, median) 1, 1	2, 2
	2, 1, 2		2, 2
	2, 2		2, 1, 2 (minute) 1, 2
VIII	2, 2	2 (long, median) 1, 1	0
	2, 2		—

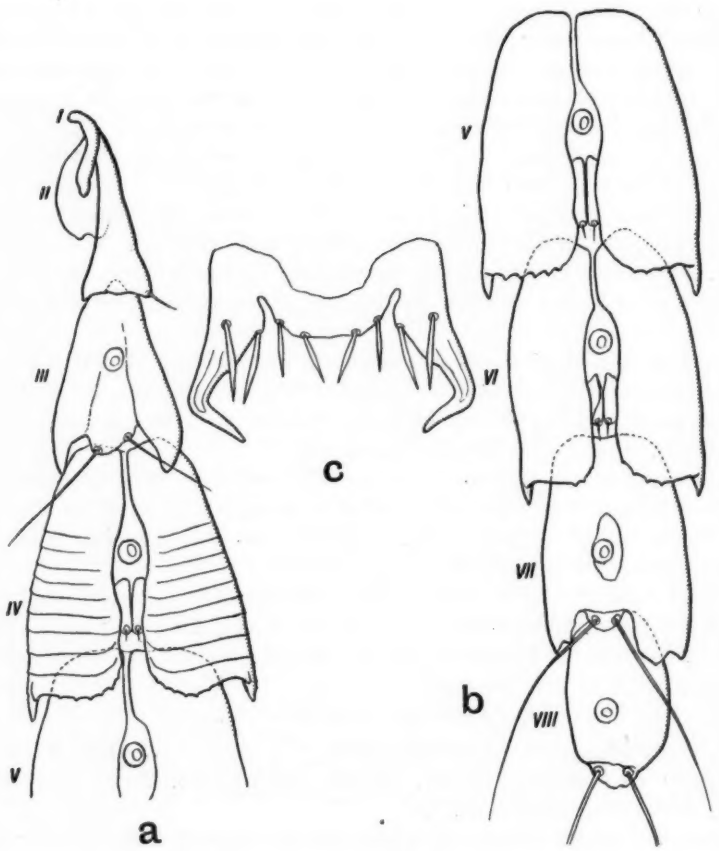

Fig. 1. *Ancistroplax crocidurae* n.g., n.sp.
a, *b* Pleurites of ♀.
c Tergite VI of ♂.

♂. Total length 1·06. Breadth (segment V) about 0·35 mm. Head as in female.

ABDOMINAL CHAETOTAXY.

Segment	Tergite	Sternite
III	4, 4	3, 3
IV–V	2, 2	1, 1, 1
	3, 3, or 2, 1, 2	3, 3, or 3, 1, 3
VI	2, 2 / 2, 2 } or 2, 2, 2, 2	1, 1 / 3, 3 }
VII	1, 1	2, 2
	1, 1	
VIII	2, 2 (in arc)	1, 1

The 7th pleurite bears medianly 2 bristles, 1 minute (dorsal), and 1 long (ventral). Pleural chaetotaxy otherwise as in female, except that the pair of bristles on the 3rd pleurite are rather short, the longer (ventral) one only just longer than the width of the flattened pleurite itself.

Holotype (♂), Allotype (♀) and Paratypes, a small series in British Museum. Host *Crocidura horsfieldi* (Tomes). Ceylon, 300 ft. W. W. A. Phillips Coll. 21. viii. 1928.

(*MS. received for publication* 14. I. 1929.—Ed.)

CREPIDOBOTHRIUM FRAGILE N.SP., A TAPEWORM OF THE CHANNEL CATFISH[1].

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(With Plate XII, including 8 Figures.)

While investigating the species of *Corallobothrium* (Essex, 1928) I examined the intestinal parasites of 180 channel catfish (*Ictalurus punctatus*) taken in Rock River (Illinois). Only six of these fish harboured tapeworms that could not be assigned to the genus *Corallobothrium*. From the six fish, besides the species of *Corallobothrium*, I found fifteen adult Cestodes belonging to the genus *Crepidobothrium*. A study of these parasites has shown that they constitute a new species for which I propose the name *Crepidobothrium fragile*, because of the ease with which the strobila is broken. The members of this genus have been reported previously only from amphibians and reptiles. This is, so far as I am aware, the first recorded occurrence of a species of *Crepidobothrium* from a fish host. Nybelin (1917) has shown that the genus *Ophiotaenia* (La Rue, 1911) is synonymous with *Crepidobothrium*. It has been pointed out by Woodland (1925, 1925 *a*) that the characters used by La Rue (1914) as the basis for the separation of the genera of Proteocephalids have been unreliable because of their inconstancy. He has proposed that all the genera of the family Proteocephalidae be placed in the genus *Proteocephalus*. Meggitt (1927) has indicated that this procedure is too sweeping and if followed would make "a most heterogeneous assemblage of some 100 species." Therefore, he has placed all these species under four genera, namely, *Corallobothrium*, *Crepidobothrium*, *Gangesia* and *Ichthyotaenia* (= *Proteocephalus*), all of which have been well established for a number of years, and greater confusion of the literature may be avoided if they are retained until some worker, in the light of more complete knowledge, again makes a revision of the entire family. Since there yet remains some doubt as to the characters that will ultimately prove trustworthy in the classification of the Proteocephalids, descriptions of new species should be as complete as possible. Although there is much variability in the measurements of a given species of Cestode, yet the fact should not be overlooked that the range of size of the organism and its parts is of considerable aid in classification. It is admitted that there is great variability in size in a given species of Cestode, but if the extremes are known the size becomes of significant value.

[1] Read before the American Society of Parasitologists, New York City, December 27, 1928 to January 2, 1929.

DESCRIPTION OF *CREPIDOBOTHRIUM FRAGILE*.

Fully matured specimens measure from 45 to 80 mm. long. The greatest diameter ranges from 1·20 to 1·90 mm. The segmentation is decidedly distinct. The proglottids are easily torn apart, so much so that extreme care must be exercised to preserve a complete individual. When the Cestode is in a contracted condition there is marked lateral overlapping of the anterior on the posterior segments. The posterior margin of each segment has a greater diameter than the anterior, giving the proglottid a crenate form. This is not so pronounced in more extended specimens. In all segments of some specimens the posterior margin is broader than the anterior, which gives to the lateral border of the chain a serrate condition (Figs. 3 and 6). In certain individuals the mature segments are somewhat ovoidal (Fig. 4). The small globose head measures about 0·5 by 0·5 mm. It is provided with four rounded suckers, each of which is situated in the middle of a fleshy prominence which is set off from the next by a more or less distinct depression (Fig. 1). Sections of the scolex reveal neither a rudimentary nor functional fifth sucker. A long distinct neck is present and measures from 5 to 10 mm. in length and from 0·45 to 0·73 mm. in width. The anterior segments of the chain are much broader than long. The posterior segments may be broader than long or longer than broad. The reproductive organs and their arrangement are typical of the genus. All of these organs, with the exception of the vitellaria, lie wholly within the well-developed inner longitudinal muscle sheath. The genital pore is marginal and irregularly alternate and lies in the first third or the second fourth of the segment. A genital papilla is absent.

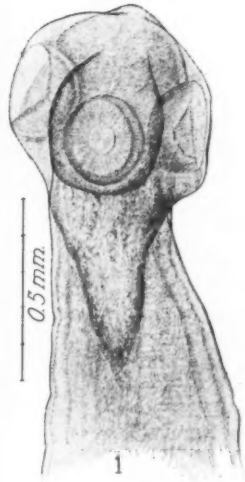
The most striking characteristic of the male organs is the great length of the unarmed cirrus. There is a marked tendency on the part of this species to evert the cirrus. Some of the specimens while living were seen with all the cirri everted, which gave the parasite the appearance of a millipede. Even after killing and fixing many of the cirri were fully extended and measured from 0·88 to 1·24 mm. long (Fig. 2). Since the length of the mature proglottid is only from 1·21 to 1·64 mm., the everted cirrus may equal half or more than half the proglottid length. When inverted the cirrus lies in many coils within the cirrus sac, which measures from 0·16 to 0·19 mm. by 0·30 to 0·35 mm. and equals from one-sixth to one-fifth the diameter of the proglottid. The cirrus empties beside the vagina into a common genital atrium. The cirrus sac, as well as the vagina, passes to the lateral margin between the dorsal and ventral excretory tubules and ventral to the longitudinal nerve cord. The vas deferens forms a large mass of coils which measures from 0·19 by 0·25 mm. to 0·25 by 0·58 mm. and extends from the level of the cirrus sac to near the median line of the proglottid. The ovoidal testes, which number from 150 to 230, lie in two fields posteriorly but coalesce anteriorly (Figs. 4 and 8). Their greatest diameter when mature is from 0·07 to 0·09 mm.

The female reproductive system is typical of the genus. From the common

genital atrium the vagina, which possesses a well-developed sphincter, passes along either the anterior or posterior border of the cirrus sac. In 101 segments selected at random from six toto mounts the vagina was anterior to the cirrus sac in fifty-seven segments and posterior in forty-four. Sections also revealed this irregularity in the position of the vagina with reference to the cirrus sac (Fig. 7). From the region of the cirrus sac the vagina passes to the median line of the segment ventral to the coils of the vas deferens, then turns abruptly posteriorly, and proceeds to the interovarian space. Throughout its extent it lies dorsal to the uterus. After making a few coils in the interovarian space it empties into the oviduct just before the latter passes over into the ootype. The two-lobed ovary lies in the posterior region of the segment more dorsally than ventrally. The lobes extend laterally to the vitellaria of each side. From the ventral region of the ovarian commissure the ova pass into the oöcapt, the oviduct leads posteriorly and, after a bend or two, turns dorsally and anteriorly. Just before it reaches the ootype it receives the vagina, already described, and the common vitelline duct. The ootype is typically Proteocephalid in character. A loose-walled duct, the uterine passage, carries the ova from the ootype anteriorly and dorsally, paralleling the vagina, and deposits them in the uterus at a point about the level of the cirrus sac. The uterus in mature specimens occupies the major portion of the medullary parenchyma. It extends from near the anterior limit of the segment to the anterior margin of the ovary; some of the diverticula often project beyond that point. Laterally the uterus usually falls short of the vitellaria. The testes are usually found crowded between the uterus and vitellaria (Fig. 5). The number of uterine diverticula ranged from seven to twelve on each side, but the usual number is from eight to ten. Along the ventral median line the uterus opens to the exterior through two or three uterine pores. The vitellaria are follicular and form two columns which extend from near the anterior to near the posterior margin of each segment. They lie along the lateral limits of the medullary parenchyma. Frequently masses of follicles project through the inner longitudinal muscle sheath and extend into the cortical parenchyma as far as the subcuticular layer of cells. The vitellaria are usually more extensive posteriorly than anteriorly. At about the level of the oocapt a duct arises from the vitellaria of each side, the two uniting near the median line to form a common vitelline duct which widens considerably, forming a vitelline reservoir, before emptying into the ootype. The ova of this species are typical of the family. The oncosphere, in preserved material, measures about 13 to 16μ in diameter.

The excretory system consists of the usual large ventral or descending ducts on each side and the small dorsal or ascending ducts. From the descending vessels small branches lead off to the ventral surface and empty through a small pore, the foramina secondaria, to the outside (Fig. 5).

Crepidobothrium fragile agrees more closely with *Crepidobothrium testudo* (Magath, 1924) than any of the other species of *Crepidobothrium*. The fol-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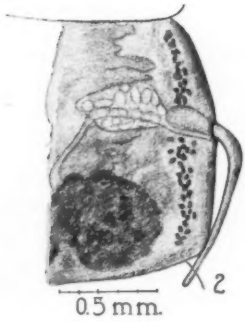
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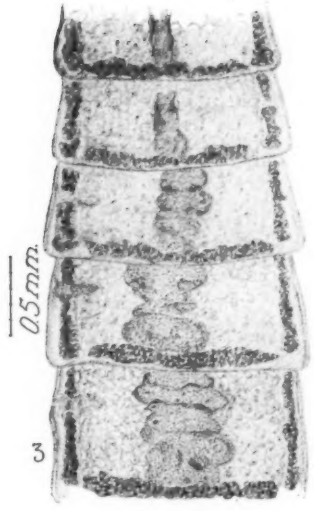
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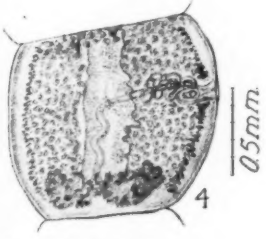
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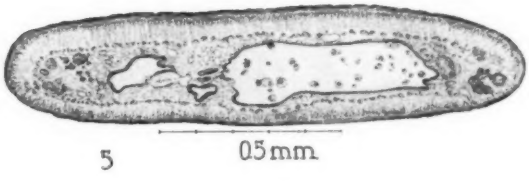
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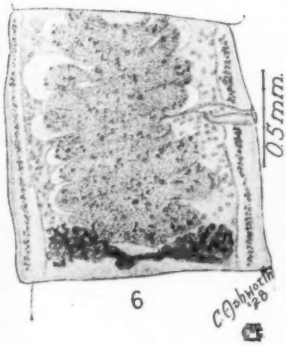
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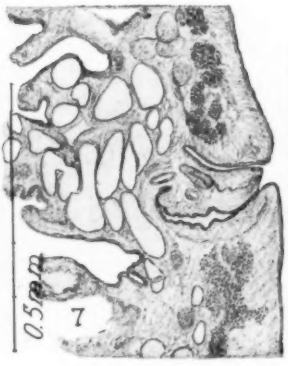
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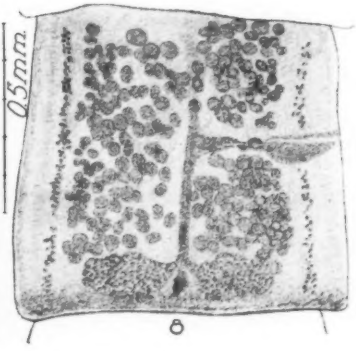
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lowing comparison, however, indicates that the two species may be easily distinguished on the basis of size of the cirrus sac, size of testes, the ratio of length of cirrus sac to width of segment, position of genital pore, and the position of the vagina with reference to cirrus pouch:

	Crepidobothrium testudo	*Crepidobothrium fragile*
Number of testes	125–200	150–230
Cirrus	Unarmed	Unarmed
Cirrus sac	0·56 by 0·27 mm. (a fourth to a third of the width of the segment)	0·16 to 0·19 mm. by 0·30 to 0·35 mm.
Genital pore	Very anterior	In second fourth or first third
Apical organ	Absent	Absent
Greatest diameter of testes	0·05 to 0·06 mm.	0·07 to 0·09 mm.
Uterine pouches	15 to 20	14 to 24
Position of vagina	Anterior to cirrus sac	Anterior or posterior

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EXPLANATION OF PLATE XII.

Fig. 1. Scolex and portion of neck.
Fig. 2. Ripe proglottid showing everted cirrus.
Fig. 3. A portion of the strobila showing immature and mature proglottids.
Fig. 4. Partially extended mature proglottid.
Fig. 5. Transection near middle of segment.
Fig. 6. Ripe proglottid well extended.
Fig. 7. Frontal section through genital atrium.
Fig. 8. Immature proglottid showing distribution of testes.

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ON SOME NEW AVIAN CESTODES FROM INDIA.

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(With Plate XIII, containing 25 figs.)

On many occasions during the years 1921 and 1922 I devoted my leisure to collecting Cestode and other Helminth parasites from wild birds in the United and Central Provinces of India. A large proportion of the material collected consists of species already known but the remainder contains some new and interesting forms, and three of these I am describing in the present paper.

Weinlandia phalacrocorax n.sp. (Figs. 1–8.)

From the Large Cormorant (*Phalacrocorax carbo*).

In the intestines of two specimens of *Phalacrocorax carbo*, which I shot on the river Barua, near Chitrakote, a village in the Banda district of the United Provinces, I found two species of Cestode: (1) a small species of *Oligorchis* and (2) three or four specimens of a much larger species which represent a very distinct and in many ways remarkable new species of Mayhew's genus *Weinlandia*. This new species is characterised by the extreme specialisation of most parts of its anatomy, especially the male genitalia and the muscular system.

Anatomy.

The maximum length of the worms is about 100–120 mm. and the maximum breadth (posteriorly) about 1·5 mm., and all the proglottids are very much broader than long. The longest proglottids are 0·1 mm. long, corresponding to a breadth of 1·3 mm. The shape of the worm (Fig. 1) is characteristic, the anterior 20–30 mm. being very slender and thread-like (with a more or less uniform breadth of about 0·16 mm.), the region behind this gradually broadening posteriorly. All my large worms are unfortunately devoid of a scolex, but I have found one very young intact specimen (only about 1·6 mm. long) and in this the scolex, though very distorted and quite undepictable, is apparently unarmed and without a rostellum. It could not have measured more than 0·33 mm. in diameter. Mature genitalia are to be found in proglottids of about half the maximum width, and it can at once be seen that the genital pores are strictly unilateral and on the left side of the strobila, and that the genital ducts run dorsal to both the excretory canals and the lateral nerve.

The testes are three in number, one testis being poral and the other two (one behind the other) aporal, the testes, in each case, lying, when viewed dorso-ventrally, in the strip of parenchyma situated between the dorsal and ventral excretory canals on the one hand, and the lateral longitudinal nerves on the other (Figs. 2, 3), *i.e.* external to the canals and internal to the nerves. This situation external to the canals is a very unusual one. The individual testes are transversely elongated and measure in surface view about 66 × 25 microns.

The cirrus sacs are also of an unusual type. Each cirrus opens on the anterior part of the margin by a small pore which opens directly to the exterior (in the small depression behind the overlapping posterior edge of the preceding proglottid), the vaginal pore being contiguous and lying immediately ventral and in the same transverse vertical plane. The cirrus itself is unarmed and eversible only to a small extent (Figs. 2, 5) and is not well-defined as apart from the vas deferens with which it is continuous, there being no line of demarcation between the two that I can observe in my preparations. I shall regard the portion of duct situated inside the outer region of the cirrus sac (next to be described) as the cirrus proper (*CIR*) and the internal continuation of this duct to the inner end of the inner region of the cirrus sac as vas deferens contained within the sac limits (*CIR'*). The cirrus sac is apparently very different from the majority of cirrus sacs of other Cestodes (*vide* Braun, 1894–1900, pp. 1410–1413), in which the whole length of the cirrus (and part of the vas deferens) is enclosed by the undivided sac and in which the musculature is both longitudinal and circular, closely associated. In the present species, the cirrus is slightly dilated next its opening and longitudinal muscles are, in this region (about 60 microns long), developed in its walls. This region I regard as the external portion of the cirrus sac (*OCS* in Fig. 4). The cirrus, or rather vas deferens (*CIR'*) then becomes narrow with non-muscular walls and continues inwards in this condition for a distance of about 0·23 mm. when its walls again dilate to form the very conspicuous internal region of the cirrus sac (*ICS* in Fig. 4), in which the musculature is wholly circular. This internal region of the cirrus sac measures about 0·26 × 0·06 mm. and its function would appear to be wholly that of driving the semen into the cirrus or vas deferens from the vesicula seminalis, while the outer region of the sac (longitudinal muscles only) is apparently wholly concerned with everting the cirrus: in other words, in this species the longitudinal and circular muscles of the sac are separated into two distinct regions instead of being combined in one in the usual manner. The vas deferens contained in the internal region of the sac may be slightly curvilinear (Fig. 6) or even convoluted.

The internal region of the cirrus sac (which lies dorsally) opens internally into another abnormally-developed structure which at first glance resembles a large transverse excretory canal but which is actually a greatly dilated long coiled vesicula seminalis. This vesicula seminalis, in transverse sections, assumes several forms (two of these are shown in Figs. 2, 4) but it always crosses

(at first towards the ventral side and anteriorly) to the aporal or right side of the proglottid (beyond the excretory canals but not so far as the aporal longitudinal nerve) and then retraces (dorsally and posteriorly) its course to the poral side, ending blindly at a point just above the left side of the ovary. In gravid segments the vesicula seminalis contains sperms but it is empty in mature segments, and it attains a maximum width of about 75 microns. From each of the three testes a very narrow (7 microns) vas efferens opens, independently of the others, into the dorsal side of the vesicula seminalis—one on the left and two on the right. The whole of the vas deferens external to the sac is thus transformed into the large vesicula seminalis.

The vagina opens to the exterior, as already stated, immediately ventral to the cirrus (and in the same transverse plane) and for the first part of its course, *i.e.* as far inwards as the beginning of the internal region of the sac, is, in transverse sections, slightly dilated; it then becomes narrow and continues so until it has passed the excretory canals when it broadens considerably and runs posteriorly and then forwards direct to the ootype on the dorsal surface of the ovary (Fig. 7). In horizontal sections the external portion of the vagina appears to be of the same breadth as the cirrus. It is devoid of a covering of gland cells.

The fully-formed ovary is large (extending laterally over nearly one-third of the proglottid breadth) and medianly situated (Fig. 3). In form it consists of two lobulated wings (alae) united anteriorly by a median isthmus, the whole being concave posteriorly to accommodate the large vitelline gland (ventral), shell gland (midway in depth of proglottid) and innermost dilated portion of the vagina (dorsal). The posteriorly situated vitelline gland is a large (cir. 128 × 60 microns in surface view) lobulated structure, which stains deeply as compared with the adjacent ovary. The shell gland is hidden in *toto* preparations by the vitelline gland below and the vagina and vesicula seminalis above, but in transverse and horizontal sections it has the usual structure of a mass of gland cells surrounding a duct lumen and measures about 73 × 51 microns in transverse and 47 × 25 microns in horizontal sections. The disposition of the ootype, vitelline duct and oviduct appears to be normal.

The uterus appears to be more or less of the usual type in its development. Even in proglottids which have not attained full maturity, thin but definite strands of tissue can be observed, in *toto* preparations and horizontal sections, to run from the unformed ovaries to the proglottid margins (passing between and dorsal to the two testes aporally and in front of and dorsal to the single testis porally) and to become slightly thickened at the marginal ends, and appear to suggest that these streaks develop initially quite apart from the ootype and only later become connected. In fully mature proglottids these strands have developed into definite tubes of about the same thickness as the vaginae, the outer extremities ending blindly just internal to the outer layer of longitudinal muscles, and the inner ends being continuous with the region of the ootype. The eggs from the ovary later enter these uterine tubes (which

become greatly dilated) and move outwards to the proglottid margins and in the meantime the outer ends of the uterine tubes have protruded into the marginal parenchyma beyond the outer muscle bundles and nerves until they lie immediately underneath the marginal subcuticula and become distended with eggs to form large projecting spherical swellings, one on each margin of each proglottid (Fig. 8). Finally, all or most of the eggs are collected in these marginal swellings and are probably liberated to the exterior by the bursting of the outer subcuticula and cuticle.

In the gravid proglottids the ovaries have for the most part disappeared, though the cirrus sacs and vaginae persist. The testes atrophy soon after the eggs enter the uterine tubes, but both pairs of excretory canals persist in the gravid segments.

The intra-uterine eggs are oval in form and have the usual three envelopes. Hooks are absent in my material. The eggs measure externally about 22 × 14 microns and the embryos about 19 × 8·6 microns.

The remaining parts of the mature proglottid to be described are the muscles, the excretory canals and the longitudinal nerves. The muscles are highly specialised, being very definite in form, number and arrangement. The two layers of longitudinal muscles are very distinct, the inner layer consisting always of eight large bundles (four above and four below), each bundle consisting of 60–100 fibres, and the outer layer consisting of 60–80 smaller, though very definite, bundles which lie immediately under (*i.e.* internal to) the equally well-developed layer of circular muscles. The circular muscle layer thus lies external to the longitudinal bundles (as in some other Hymenolepids) and the latter are therefore "medullary" in position (Fig. 2).

The usual two pairs of excretory canals are present. Each pair lies internal to the row of testes of its side and the dorsal canal lies immediately dorsal to the ventral, no, or very little, space intervening. Both canals are fairly thick-walled, the dorsal more than the ventral. So far as I can determine, the four canals never communicate with each other in the strobila region, the transverse canals, so often present in other species, being absent. The ventral canals measure about 44 microns in external diameter and the dorsal canals 18 microns.

The lateral longitudinal nerves are well marked, lie internal to the outer layer of longitudinal muscles and are therefore medullary in position.

SYSTEMATIC POSITION.

The great breadth of the proglottids as compared with their length, the unarmed scolex and absence of a rostellum, the passing of both genital ducts dorsally to the excretory canals and nerve and the three envelopes enclosing the eggs at first led me to suppose that the present worm belonged to the family of the Anoplocephalidae (in some species of which the testes also are very reduced in number) and it was not until Dr Southwell kindly pointed out that the presence of three testes indicated the genus *Hymenolepis* that I came to

realise that the characters just enumerated were equally applicable to the family Hymenolepididae. In truth the two families (and the Davaineidae?—a family solely based on its scolex hooks: *vide* Mayhew 1926, p. 10) merge into each other and the only justification in many cases for referring a worm to one or the other is mere precedent. In the present instance the further characters of unilateral pores and lack of paruterine organs and egg-capsules compel us, for the same reason, to refer the worm to the Hymenolepidinae (just as these same characters, on my previous supposition, compelled me to refer it to the Anoplocephalinae). Of the nine genera included by Mayhew (1926) in the Hymenolepidinae, six contain three testes per proglottid and the present worm, judging only by the arrangement of the three testes and the other characters named by Mayhew (p. 52), should be referred to *Weinlandia*. Mayhew, however, points out, in the case of *Hymenolepis microcephala* (p. 42), that the position of two of the testes external to the excretory canals "stands in such marked contrast to the other species of the *Hymenolepis* group that a more careful study may result in the placing of this species in a separate genus" and if this suggestion is to be followed for a species with two testes in this peculiar position, it must also be followed for a species with all three testes so situated. But this character is, in my opinion, not sufficiently definite nor fundamental to justify the creation of a separate genus and I prefer to regard the present worm as a well-marked species of *Weinlandia*, Mayhew 1926, with the following designation and diagnosis:

Weinlandia phalacrocorax.

Maximum length of worm about 120 mm. Anterior quarter or third of strobila very slender, and all segments very broad in proportion to their length. Genital pores on left side of strobila. All three testes situated external to the excretory canals. Cirrus sacs large and each is divided into a small external portion provided only with longitudinal muscles for the exsertion of the cirrus and a large internal portion provided only with circular muscles for the propulsion of the semen. Cirrus unarmed. The whole of the vas deferens outside the cirrus sac is converted into a vesicula seminalis which is long and wide and coiled on itself in the dorsal region of the medulla, ending blindly ventral to the internal portion of the sac and receiving the three vasa efferentia dorsally. The vagina opens ventral to the cirrus sac and lies ventral to and in the same transverse plane as the sac, is dilated internally (bending dorsally) and is devoid of a covering of gland cells. Ovary large and consists of two alae united by an antero-ventral median isthmus. Vitelline gland large, postero-ventral and median. Large median shell gland lying ventral to the inner end of the vagina and dorsal to the isthmus. Longitudinal muscle bundles in two concentric layers, the inner consisting of four bundles above and four below and the outer layer consisting of 60–80 smaller bundles lying immediately internal to the circular muscle layer. Two pairs of excretory canals, both thick-walled, the smaller dorsal lying immediately dorsal to the ventral. Transverse excretory

canals are absent. Lateral longitudinal nerves well marked and lie internal to the outer layer of longitudinal muscle bundles. Gravid uterus forms large marginal swellings filled with eggs. Uterine eggs measure externally about 22 × 14 microns and are hookless. Host: *Phalacrocorax carbo*, the Large Cormorant of India.

Malika oedicnemus gen. et sp. nov. (Figs. 9–15.)

Oedicnemus scolopax, the Stone Curlew, commonly occurs near Allahabad in the United Provinces, and in the intestines of a number of specimens I have discovered two species of tapeworm: (1) a small species of Meggitt's genus *Prochoanotaenia* (possibly the *Taenia coronata* of Creplin 1829 and the *Choanotaenia coronata* of Fuhrmann 1908) found in the duodenum and anterior region of the intestine, and (2) a much larger species found in the middle and hind regions of the intestine now to be described. This new species, as we shall see, constitutes the type of what most authorities would consider to be a well-marked new genus and I have therefore named it after the small village situated near Allahabad which often served as my headquarters.

ANATOMY.

The worms attained a length of about 60 mm. and a maximum breadth of about 1·6 mm. All the segments are broader than long, save occasionally a terminal segment. Mature (*i.e.* with formed genitalia previous to the development of the uterus) unflattened segments measure approximately 1·5 mm. × 0·05 mm. and so are quasi-Anoplocephaline in form; gravid proglottids are distinctly longer relative to their breadth, measuring on the average about 1·2 mm. × 0·4 mm.

The scolex (Fig. 9) is about 0·5 mm. broad and about 0·4 mm. long. The suckers measure, when viewed sideways, about 183 × 135 microns. The rostellum is the usual robust muscular structure measuring, when retracted, 0·25 mm. in length, and bearing a single circle of about 30 hooks, each measuring about 73 microns in length (Fig. 10). There is no unsegmented neck region.

In the strobila the genital openings are seen to be unilateral (on the left side) and to open in each proglottid a short distance in front of the middle point of the margin (Figs. 11, 14). In mature segments (Fig. 11) the arrangement of the genitalia is as follows. The testes are about 18–27 in number, are small and lie in a transverse row, two to three layers deep, towards the hind border of the segment and behind the ovary and vitelline gland. In transverse sections the testes are seen to lie about midway between the upper and lower surfaces of the medulla and to measure about 88 × 55 microns. The atrium opens on the margin by a small aperture which leads immediately into a large and conspicuous chamber, into the base of which open both the cirrus sac and the vagina. The cirrus sac lies in front of the vagina and is about 0·2 mm. in length and 0·04 mm. in maximum breadth, reaching as far as the ventral excretory canal. The cirrus is unarmed and the portion of vas deferens inside the

sac is straight, save at the inner end of the sac where it bends over and becomes dilated before leaving the sac. Outside the sac the vas deferens forms an elongated mass of coils lying anteriorly in the segment in front of the ovary. The vagina is a narrow tube which runs directly to the ootype with only one or two very slight dilatations at its inner end (receptaculum seminis) and is devoid of a covering of gland cells. Both the male and female ducts run between the dorsal and ventral excretory canals and dorsal to the nerve on the left side of the strobila. The ovary is distinctly poral and slightly anterior in position and in surface view is of the form shown in Fig. 11. The vitelline gland is compact but irregular in form and is median and slightly posterior in position. My preparations are not sufficiently good to enable me to describe the shell gland and finer ducts.

The medulla is separated from the cortex by a thin layer of transverse muscles, outside which lie an inner layer of large bundles (about 12–20 fibres in each) of longitudinal muscles, another very thin layer of transverse fibres, and an outer layer of small bundles (2–4 fibres in each) of longitudinal muscles, which lies next the subcuticular layer. A single layer of minute longitudinal fibres underlies the cuticle. The usual two pairs of longitudinal excretory canals are present, the ventral being much larger than the dorsal. The longitudinal nerves lie just internal to the inner layer of longitudinal muscles.

The uterus (Fig. 13) develops as a number of lateral branched slender outpushings from the ootype region with dilated extremities full of eggs, extending as far outwards as the ventral canals and finally occupying the whole of the medulla. These outpushings ultimately, in gravid segments, become separated off and form closely-packed capsules, each containing a number (about 3–13) of oncospheres (Fig. 14). The oncospheres are slightly elongated and measure (in balsam) about 62 microns in length and 22 microns in breadth (Fig. 15). The hooks measure about 30 microns in length. Each oncosphere is invested by a thin sheath.

Systematic position.

This worm is of course a Hymenolepid and belongs to the subfamily Dipyliinae in virtue of its uterus becoming replaced by egg-capsules. If, however, we consult a key to the genera of the Dipyliinae (*e.g.* that of Meggitt) it becomes evident that this new species will not fit the definition of any existing genus, since the only existing genus which, like the present species, has a single set of genitalia in each proglottid, in which the genital ducts pass between the excretory canals and has unilateral genital pores, is *Eugonodaeum*, and this genus is further characterised by having an unarmed rostellum, few testes, no uterus and one egg in each capsule—features not possessed by the present species. Since it is the universal practice to use such characters as armed or unarmed scolices, number and position of testes, mode of development of the uterus and the number of eggs in the capsule for defining genera, no alternative remains save to propose another new genus to contain the present species.

The following characters are diagnostic of the new genus *Malika*:

Dipyliinae. Rostellum armed. All proglottids (save occasionally the terminal segments) considerably broader than long. Genital pores unilateral. Genital ducts pass between the excretory canals. A single set of genitalia in each segment. Ovary poral. Sacciform uterus which resolves into capsules, each containing several eggs. Adults in birds.

This new genus is thus essentially distinguished from *Eugonodaeum* by the possession of an armed rostellum, a temporary sacciform uterus and capsules each containing several eggs. The type species is *M. oedicnemus*, from *Oedicnemus scolopax*, India.

Possibly the species recently described by me (Woodland 1928) from *Numida ptilorhyncha* of the Sudan and which I left unnamed, may belong to this genus, since it only essentially differs, as regards known characters, in the forms of the suckers and hooks, the number of testes and absence of the dorsal excretory canal, but we have yet to ascertain the development of the uterus and the disposition of the eggs in the gravid segments. Should this as yet unnamed species prove to belong to *Malika*, its name should obviously be *M. numida*.

Cameron (1928) has recently described a new Cestode from the Rusty Tiger Cat—*Felis planiceps*—which only differs slightly from Johnston's *Similuncinus dacelonis* (1909; the host is the "Laughing Jackass") in the number (12 in the former and 36 in the latter) and form of the scolex hooks, the number of testes, position of coils on the vas deferens, presence or absence of seminal vesicle and forms of ovary and vitelline gland, and on these distinctions he proposes a new genus *Aleurotaenia*. Personally I think that if we allow such relatively trivial differences as these to serve for generic distinction, classification will soon arrive at a state of hopeless confusion. *Malika*, on the other hand, owes its origin to three characters which, according to present practice, are weighty compared with those distinguishing *Aleurotaenia*. It is true that "*Aleurotaenia*" is from a mammal and *Similuncinus* from a bird and these two Cestodes may have had very divergent origins and therefore in reality be super-generically distinct, but classification, from the systematist's standpoint, cannot, in the absence of positive evidence, take possible convergence of characters into account in its definitions, and, in my opinion, Cameron's *Aleurotaenia planicipitis* must lapse into *Similuncinus planicipitis*. I cite this example of an allied genus side by side with the new genus *Malika* to illustrate the necessity of imposing some lowest limit (undefinable though it may be) on the value of characters essential for generic distinction.

Rhabdometra dendrocitta n.sp.

From *Dendrocitta rufa*.

So far, seven species of *Rhabdometra* have been described, all from Galliform birds save *R. melierax* from a Sudanese Hawk—*Melierax gabar* (Woodland 1929). The present new species is therefore the eighth to be known and is

also from a non-Galliform bird, viz. *Dendrocitta rufa*, the common Indian Tree-Pie, found in the plains of India. I possess numerous examples of this species obtained from birds shot by me near Allahabad.

The worms (mounted unflattened in balsam) vary in length between 40 mm. and 60 mm. and have a maximum breadth (posteriorly) of about 1 mm. The individual proglottids are rather contractile and are usually broader than long but not necessarily so. Anteriorly (near the scolex) the immature proglottids have a breadth of about 0·5 mm. and a length of only 0·03 mm. Mature proglottids are also very short, but the gravid proglottids are decidedly longer and towards the end may be twice as long as broad and with the posterior margin much expanded (Fig. 24). The scolex (Fig. 16) is broader (0·6–0·8 mm.) than the succeeding strobila and very short (about 0·02 mm.), the large suckers practically occupying its entire length. There is no trace of a rostellum or of hooks or spines. An unsegmented neck is entirely absent, strobilisation extending up to the lower level of the suckers.

Rudiments of the genitalia appear in the proglottids almost immediately behind the scolex and mature genitalia are to be found at less than 10 mm. from the scolex. The testes (Fig. 18) are situated dorsally in a median group and are about 10–12 in number. The testes measure about 58 × 25 microns in transverse sections. The genital ducts are irregularly alternating, pass between the dorsal and ventral excretory canals and ventral to the nerves, and open towards the anterior ends of the margins. The cirrus sac (Fig. 17) is relatively small (cir. 0·16 mm. long) and simple in structure (the cirrus being only slightly twisted) and opens in front of the vagina into a very small atrium. The vas deferens, on emerging from the sac, forms a large conspicuous mass of coils surrounding a large retractor muscle (not shown in Fig. 17). The vagina is a narrow tube which runs directly to a small oval receptaculum seminis and thence to the large sac-shaped ovary which lies slightly porally. At the aporal end of the ovary lies the spherical vitelline gland. My preparations are not sufficiently clear to enable me to detect the shell gland or the finer ducts associated with the ovary.

In the later stages of development (Figs. 19, 20) of the proglottid the ovary becomes more distinctly an ovoid sac, with a limiting wall and containing large separate eggs, and this sac is the uterus of gravid segments—the ovary apparently becomes directly transformed into the uterus. The vitelline gland gradually dwindles before disappearing and the testes also degenerate. At the aporal end of the ovary or rather uterus a paruterine organ develops (Fig. 19), at first as a small patch of fibrous tissue situated between the degenerate vitelline gland and the uterus. Later the rudiment of the paruterine organ lengthens (transversely, though the fibres run antero-posteriorly) in the narrow proglottids (Fig. 20). As the proglottids lengthen, the uterus becomes disposed at right angles to its initial position, *i.e.* with its length parallel with the length of the strobila, and the paruterine organ appears as a closely-attached ovoid mass on its aporal side (the vesicula seminalis still persisting on its poral side),

as shown in Fig. 20. At a still later stage of development, the vesicula seminalis and the remainder of the genital ducts disappear, and the paruterine organ enlarges and projects into the uterus (Fig. 21). Finally the paruterine organ develops a cavity continuous with that of the uterus (Fig. 22) and the eggs in the uterus migrate into the paruterine organ (Fig. 23), which ultimately contains all the eggs (the uterus being absorbed) and stretches, as an elongated sac, the entire length of the proglottid. In the terminal elongated gravid segments the paruterine organ alters in shape so as to project as a spherical sac beyond the posterior border of the proglottid (Fig. 24), and the embryos are probably liberated to the exterior by the bursting of this sac. The eggs, as seen *in utero* in transverse sections, are spherical and about 9 microns in diameter but later the eggs develop into elongated embryos, about 55 microns in length and 9 microns in breadth and pointed at both ends (Fig. 25). These embryos are multinucleated and possess a definite covering sheath and the usual six hooks in the middle region.

In transverse sections it can be seen that the medullary parenchyma is delimited from the cortical by a very thin layer of transverse muscle fibres, outside which lie two layers of bundles of longitudinal muscles (5–22 fibres in the larger inner bundles). There are the usual two pairs of longitudinal excretory canals, the larger ventral canals being connected transversely in each proglottid by a transverse canal at the hind border.

Though it is necessary to allocate the present species to the genus *Rhabdometra*, yet it must be admitted that as regards both the general disposition of the genitalia and the form of particular organs it differs from all other species of the genus. The principal cause of this difference is that the mature proglottids are much more narrow (antero-posteriorly of course) than in other species and there is therefore no space for the testes to be situated behind the ovary complex: on the contrary, all the testes lie dorsal to the complex. The number of testes also is very small and the ovary disproportionately large and slightly poral in position. On the other hand, the cirrus sac has the retractor muscle found in most other species and the general arrangement of the muscles, genital ducts and excretory canals is the same. I was unable, in my preparations, to ascertain the exact mode of development of the uterus. It appears to arise directly from the ovary, this becoming enclosed in a definite membrane and the eggs growing and developing shells *in situ*, but I cannot definitely affirm that this is the case. In some other species, *e.g.* in *R. nullicollis* Ransom and *R. similis* Ransom, it is stated to develop anteriorly to the ovary, while in *R. cylindrica*, Beddard says that it appears suddenly and already well developed. Further, again possibly owing to the short proglottids, the uterus and paruterine organ do not become continuous longitudinally in the middle line but remain side by side, the former gradually becoming absorbed into the latter. Finally, the embryos are very elongated and nematodiform and enclosed in a definite loose sheath, although retaining the usual six hooks.

The diagnosis is as follows:

Rhabdometra dendrocitta.

Paruterininae. Scolex devoid of a rostellum. Neck absent. Mature proglottids much broader than long. Testes few in number (about 12) and situated in a cluster lying dorsal to the ovary complex. The genital ducts open a little anteriorly to the middle of the proglottid margin and pass ventrally to the longitudinal nerves. The cirrus sac is small (cir. 0·16 mm. long) and attached to a long retractor muscle stretching across the proglottid, and the vas deferens forms a conspicuous vesicula seminalis. The vagina is devoid of gland cells and bears a small ovoid receptaculum. The ovary is large and poral in position, and apparently becomes transformed directly into the uterus. The paruterine organ develops lateral to the uterus, the latter becoming gradually absorbed into the former to form an elongated sac extending over the entire length of the proglottid. The eggs are at first spherical but later produce hooked embryos elongated in form and enclosed in a sheath.

Host: *Dendrocitta rufa*, India.

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EXPLANATION OF PLATE XIII.

REFERENCE LETTERS.

AT, genital atrium. *BE*, blind end of vesicula seminalis. *CIR*, cirrus. *CIR'*, vas deferens contained within the two divisions of the cirrus sac. *CIRC*, circular muscle layer lying external to the longitudinal muscles. *CLM*, longitudinal muscles of outer region of cirrus sac. *CM*, circular muscles of inner region of cirrus sac. *CS*, cirrus sac. *DV*, dorsal excretory canal. *GD*, genital duct. *ICS*, inner region of cirrus sac. *IL*, inner layer of longitudinal muscle bundles. *MSW*, marginal swellings of uteri for storage and liberation of eggs. *N*, nerve. *OCS*, outer region of cirrus sac. *OL*, outer layer of longitudinal muscle bundles. *OV*, ovary. *PAR*, paruterine organ. *RS*, receptaculum seminis. *SHG*, shell gland. *TES*, testis. *UT*, uterus. *VAG*, vagina. *VE*, vas efferens. *VES*, vesicula seminalis. *VIT*, vitelline gland. *VV*, ventral excretory canal.

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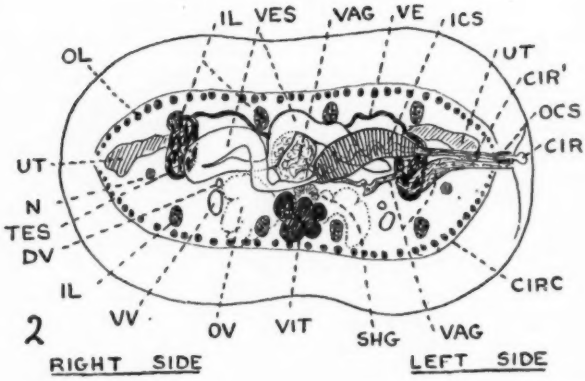
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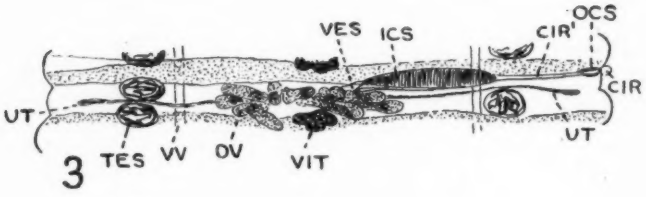
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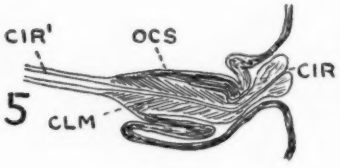
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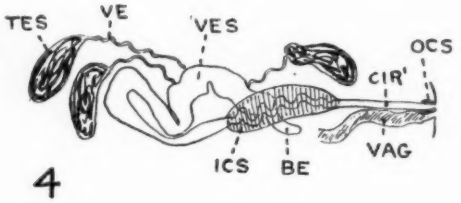
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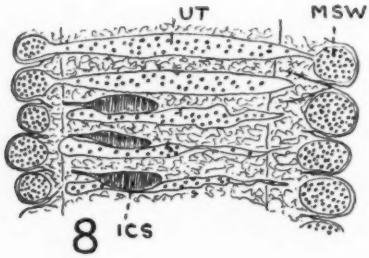
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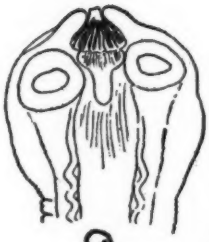
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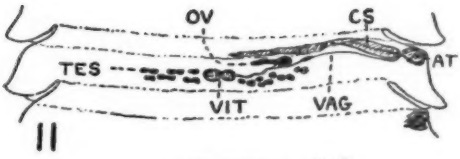
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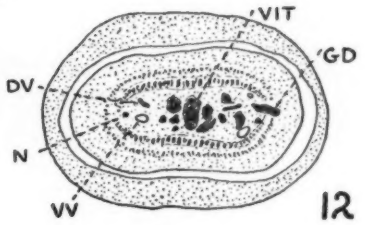
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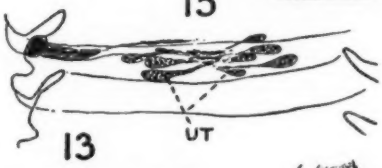
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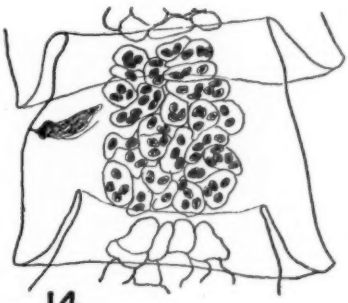
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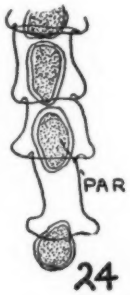
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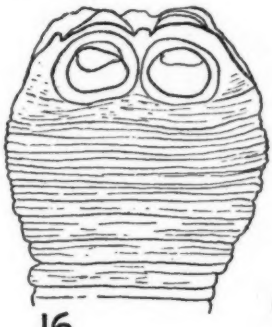
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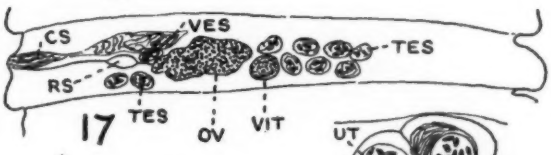
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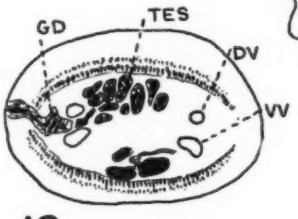
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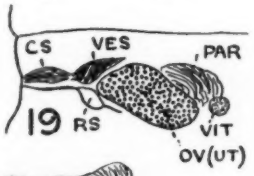
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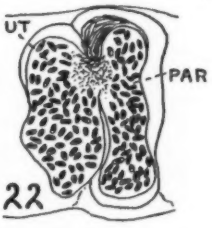
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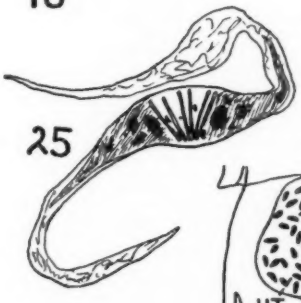
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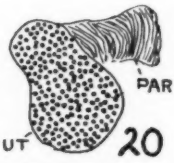
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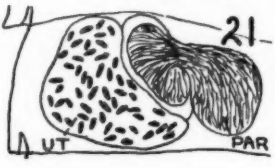
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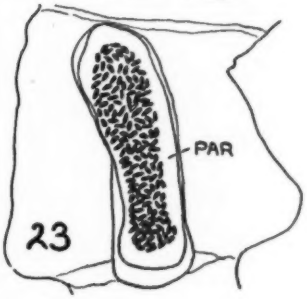
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Figs. 1–8. *Weinlandia phalacrocorax.*

Fig. 1 (approximate natural size): outline of mature worm. Fig. 2 (×56): composite drawing of transverse section of mature proglottid from the anterior aspect. The ovary is only shown in outline. Fig. 3 (×56): artificially-flattened *toto*-preparation of mature proglottid viewed ventrally. Fig. 4 (×56): semi-diagrammatic sketch of the male reproductive system seen in transverse sections. The testes are shown slightly displaced. The course of the vesicula seminalis is different from that shown in Fig. 2 and great variability is shown in different proglottids. Fig. 5 (×260): outer region of the cirrus sac as seen in a horizontal section. Fig. 6 (×260): portion of the inner region of the cirrus sac as seen in a horizontal section. Fig. 7 (×56): the vagina as seen in transverse sections. Fig. 8 (×39): portion of a horizontal section through gravid proglottids in the dorsal region (the right hand margin of one of the proglottids has apparently failed to develop a swelling).

Figs. 9–15. *Malika oedicnemus.*

Fig. 9 (×56): scolex in outline, with retracted rostellum. Fig. 10 (×160): hook from rostellum. Fig. 11 (×39): mature proglottid in ventral view from a flattened preparation. Fig. 12 (×28): transverse section of a mature proglottid. Fig. 13 (×28): young gravid proglottid in dorsal view. Fig. 14 (×39): gravid proglottid showing egg capsules. Fig. 15 (×395): hexacanth embryo.

Figs. 16–25. *Rhabdometra dendrocitta.*

Fig. 16 (×39): scolex in outline. Fig. 17 (×56): mature proglottid (from much flattened preparation). Fig. 18 (×56): transverse section of mature proglottid. Fig. 19 (×56): the first appearance of the paruterine organ on the aporal side of the ovary (future uterus) in a flattened preparation. Fig. 20 (×56): the uterus has become enlarged and elongated anteroposteriorly. Fig. 21 (×56): the paruterine organ has enlarged and is indenting the uterus. Fig. 22 (×39): the paruterine organ has developed a cavity which has become continuous with that of the uterus and eggs are passing from the latter to the former. Fig. 23 (×39): the paruterine organ has altogether replaced the uterus. Fig. 24 (×18): terminal segments (unflattened) showing paruterine organs (filled with embryos) apparently capable of being extruded posteriorly from the segments. Fig. 25 (×530): elongated hexacanth embryo with sheath.

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ON THE EFFECT OF RANDOM OVIPOSITION ON THE ACTION OF ENTOMOPHAGOUS PARASITES AS AGENTS OF NATURAL CONTROL.

By W. R. THOMPSON, Ph.D., D.Sc.

(*Imperial Bureau of Entomology.*)

In 1924 I published[1] a study of the effect of random oviposition on the development of a population of entomophagous parasites at the expense of their hosts. The present paper deals with an aspect of the matter which I neglected to consider.

If a female parasite is unable to distinguish unparasitised individuals of the host species and individuals already parasitised, either by itself or by other females of its species, and if all individuals of the host species are equally accessible to attack, the eggs of the parasite will then be distributed among its hosts according to the laws of chance.

Thus, suppose that a parasite is depositing 100 eggs in a host population comprising 100 individuals. When the first egg has been deposited, one of the hosts will, of course, be parasitised. The second egg may, however, be deposited either in the parasitised host or in one of the 99 unparasitised individuals, the chances being, of course, in favour of the latter event. As the number of parasitised individuals increases, the probability that eggs will be deposited in specimens already attacked becomes greater. If the number of individuals of the host species be $= N$, the number of eggs of the parasite be $= X$, and the proportion of hosts parasitised be $= Y$, the relation between these values is represented by the equation

$$Y = N\left(1 - e^{-\frac{X}{N}}\right) \qquad \text{.....(1),}$$

e being the incommensurable number 2·71828..... .

This equation is strictly speaking valid only when the quantities represented by X and N are very large, but as a general first approximation it may be considered sufficiently accurate for practical purposes.

I have shown in previous papers, that if the initial number of hosts be $= n$, the initial number of parasites be $= p$, the multiplication of the host per generation $= h$ and the multiplication of the parasite $= s$; and if the sex ratios are the same in parasite and host, then, neglecting other factors, the number of hosts in the generation will be

$$H_t = nh^t - psh\left[\frac{s^{t-1} - h^{t-1}}{s - h}\right] \qquad \text{.....(2),}$$

[1] *Annales de la Faculté des Sciences de Marseille*, IIe Série, T. II, Fas. II, 1924.

the number of parasites will be

$$P_t = ps^t \qquad \text{.....(3).}$$

The number, t, of generations required for the extermination of the hosts by its parasite will be

$$t = \frac{n}{p} \qquad \text{.....(4),}$$

if the reproductive rates of host and parasite are equal,

$$t = \frac{\log\left\{\dfrac{n\alpha - n + p\alpha}{p\alpha}\right\}}{\log \alpha} \qquad \text{.....(5),}$$

if the reproductive rate of the parasite is greater than that of the host, so that we have

$$s = \alpha h \quad \text{and} \quad \alpha > 1,$$

and

$$t = \frac{\log\left\{\dfrac{p}{n + p - n\alpha}\right\}}{\log \alpha} \qquad \text{.....(6),}$$

if the reproductive power of the parasite is less than that of the host, so that we have $\alpha s = h$ and $\alpha > 1$.

These formulae are constructed on the hypothesis that the parasite deposits only a single egg in each host attacked and never oviposits in any host already parasitised by its species. We have, however, no reason whatever to suppose that parasites can distinguish between parasitised and unparasitised hosts, that they are capable of choosing between these two categories of individuals or that they attack only unparasitised hosts. On the contrary, everything seems to indicate that the distribution of the eggs or larvae of parasites among the accessible individuals of the host species occurs simply according to what we rather paradoxically call the laws of chance. The effect of this random distribution of offspring on the development of the parasitic cycle and on the time required for the extermination of the host by its parasite were studied in the paper referred to above. It was there shown, that if μ represents the relation between parasites and hosts at the end of the first generation $\left(\mu = \dfrac{ps}{nh}\right)$, μ_1 this relation at the end of the second generation and μ_t at the end of the tth generation, we can write

$$\mu_t = \mu_{t-1}\, \alpha e^{\mu_{t-1}} \qquad \text{.....(7),}$$

$$H_t = n_{t-1}\, h e^{-\mu_{t-1}} \qquad \text{.....(8),}$$

$$P_t = ps^t \qquad \text{.....(9).}$$

When we compare the figures given by these formulae with those given by formulae (2)–(6), we find that the difference is relatively slight. In general, one generation must be added to the figure given by formula (5) if the parasite reproduces more rapidly than the host, and two or three generations to the figure by formula (4) if the parasite reproduces at the same rate as the host.

These conclusions depend, however, upon the assumption that no mortality results from superparasitism; or, in other words, that, no matter how many individuals accumulate in a given host, all are able to complete their development and emerge. Prof. William C. Cook, of the University of Montana, has reminded me that this assumption is questionable; and that very often the simultaneous occurrence of several larvae of a parasite in a single individual of the hosts often results in the death of many or all of them.

The actual result of the deposition of X eggs in N hosts will obviously depend upon the precise distribution of the eggs and upon the effect produced on the development of the parasite concerned of an encounter with one, two or more individuals of the same species. When only a few individuals fall into the same host, all may develop successfully. When many fall into the same host, all may die or emerge in a condition so weakened that they are unable to reproduce. As a first approximation to the facts we may, however, assume that the usual event is one intermediate between the extremes just mentioned; and that *one* parasite issues from *each* parasitised host, no matter how many eggs have been deposited therein.

Making this assumption and using the symbols referred to above, we may then proceed as follows:

In the first generation we shall have

$$n \times h = nh \text{ hosts, and}$$
$$p \times s = ps \text{ parasites.}$$

The hosts emerging at the end of the first generation according to formula (1) will therefore be

$$n_1 = nhe^{-\mu}.$$

The parasites emerging will be equal in number to the hosts killed and will be

$$p_1 = nh\,(1 - e^{-\mu}).$$

In the second generation we shall have

$$nh\,.\,he^{-\mu} \text{ hosts, and}$$
$$nhs\,(1 - e^{-\mu}) \text{ parasites.}$$

We have

$$\mu_1 = \frac{nhs\,(1 - e^{-\mu})}{nh\,.\,he^{-\mu}}$$
$$= a\,(e^{\mu} - 1).$$

The hosts emerging at the end of this generation will be

$$n_2 = nh\,.\,he^{-\mu}\,e^{-\mu_1} = n_1\,he^{-\mu_1}.$$

The parasites emerging will be

$$p_2 = nh\,.\,he^{-\mu}\,(1 - e^{-\mu_1})$$
$$= n_1 h\,(1 - e^{-\mu_1}).$$

Similarly, in the third generation we shall have

$$n_1 h\,.\,he^{-\mu_1} \text{ hosts, and}$$
$$n_1 hs\,(1 - e^{-\mu_1}) \text{ parasites.}$$

We have $$\mu_2 = \alpha\,(e^{\mu_1} - 1).$$

The hosts and parasites emerging at the end of this generation will therefore be

$$n_3 = n_2\,he^{-\mu_2},$$
$$p_3 = n_2 h\,(1 - e^{-\mu_2}).$$

Without proceeding further, it is obvious that we can write

$$\mu_t = \alpha\,(e^{\mu_{t-1}} - 1) \qquad \text{.....(10),}$$
$$n_t = n_{t-1}\,he^{-\mu_{t-1}} \qquad \text{.....(11),}$$
$$p_t = n_{t-1}\,h\,(1 - e^{-\mu_{t-1}}) \qquad \text{.....(12).}$$

There is apparently no simple formula to be derived from any of these equations permitting the direct calculation of the number of generations required, in any given case, for the extermination of the host by the parasite. The indirect calculation of this value, though sometimes tedious, is, however, quite an easy matter.

Before proceeding with any numerical examples, it is worth while making a brief study of the formulae (10), (11) and (12) since this brings out several points of interest.

Taking the equation (10) and writing $\frac{p}{n} = \kappa$ and $\frac{s}{h} = \alpha$, we have $\mu_{t-1} = \kappa\alpha$, if μ_{t-1} is the relation between parasite and host populations at the end of the first generation. Our equation (10) thus becomes

$$\mu_1 = \alpha\,(e^{\kappa\alpha} - 1);$$

we have also $$\mu = \kappa\alpha.$$

Now the possibility of control depends upon the ratio

$$\mu : \mu_1 \text{ or } \kappa : (e^{\kappa\alpha} - 1),$$

which can be written, $$\kappa\alpha : \log_e (\kappa + 1) \qquad \text{.....(13).}$$

If we have $\kappa\alpha > \log_e (\kappa + 1)$, the parasitism will rise from generation to generation and control will occur; if we have $\kappa\alpha = \log_e (\kappa + 1)$, the percentage of parasitism will remain constant and control will not occur; if we have $\kappa\alpha < \log_e (\kappa + 1)$, the percentage of parasitism will fall from generation to generation.

It has been shown in an earlier paper that even if there is no loss of efficiency through superparasitism, a parasite with a reproductive rate inferior to that of the host can never control it, unless its initial population is relatively large compared with that of the host. For example, if the reproductive rate of the host is 1·5 times that of the parasite, there must be more than half as many parasites as hosts; if the reproductive rate of the host is twice that of the parasite, there must be more parasites than hosts; if the reproductive rate of the host is three times that of the parasite, there must be more than twice as many parasites as hosts, if control is ever to occur.

The mortality and loss of efficiency due to superparasitism still further decreases the value of parasites whose effective rates of reproduction are lower than those of their hosts. Thus, in the three cases mentioned where we have

$\alpha = 1{\cdot}5$, 2 and 3 respectively, we find by varying the values of κ, that the parasite population must be more than 1·14, 2·6 and 6 times as great as that of the host, if control is ever to occur.

If the *initial populations* of host and parasite are equal, the value of α must not be less than 0·693, approximately, if the parasite is to overtake the host; if it is exactly 0·693, the percentage of parasitism will remain constant; if it is less, the percentage of parasitism will fall from generation to generation.

If the *reproductive rate* of the parasite is equal to that of the host, the ratio (13) becomes

$$e^{\kappa} : (\kappa + 1) \qquad \text{.....(14).}$$

Evidently we have $e^{\kappa} > \kappa + 1$ for all values of κ. Thus, whatever be the initial ratio between the populations of the parasite and the host, control will ultimately occur, other things being equal, if the reproductive rate of the parasite is equal to that of the host. If the reproductive rate of the parasite is greater than that of the host, control will, *a fortiori*, be certain.

Consider now the equation

$$n_t = n_{t-1}\, h e^{-\mu_{t-1}}.$$

This can be written

$$\log_e \frac{n_t}{n_{t-1}} = \log_e h - \mu_{t-1}.$$

If the host is simply maintaining itself without increasing, we shall have $h = 1$. In that case our equation becomes

$$\log_e \frac{n_t}{n_{t-1}} = -\mu_{t-1},$$

from which we deduce that the host population, in this case, steadily decreases from generation to generation. If h is greater than 1, let us suppose that it has a value of 1·2, which means a reproductive rate slightly superior to that of the host. Our equation becomes

$$\log_e \frac{n_t}{n_{t-1}} = 0{\cdot}18 - \mu_{t-1}.$$

Now in practical operations, the value of μ_{t-1} (*i.e.*, the ratio between the populations of the parasite and the host at the end of the first generation) will be, in general, far inferior to 0·18. The value of $\log_e \frac{n_t}{n_{t-1}}$ will thus be positive until μ attains the value of 0·18, after which it will be negative. In other words, the host population will increase from generation to generation until the ratio of parasites to hosts = 0·18, after which it will steadily decrease until the infestation is practically wiped out. The same thing applies for any value of h, whatever be the value of the other factors, unless they are such that μ decreases, as it will, except under certain conditions already defined, when the reproductive rate of the parasite is less than that of the host. In the latter case the value of $\log_e \frac{n_t}{n_{t-1}}$ will remain positive, and tend to the value of $\log_e h$; the host population will increase from generation to generation.

If the reproductive rate of the parasite is = 1, then, because of the mortality due to superparasitism, the parasite population will diminish from generation to generation. If the initial number of parasites is very small as compared to the number of hosts, the loss will, however, be imperceptible at first, and will, in fact, become apparent only after the percentage of parasitism is fairly high. The curve for the percentage of hosts parasitised will thus resemble at first the curve which would be obtained, if the parasite chose only the unparasitised hosts, and will differ from it only in its terminal portion. The same thing has already been noted in the case studied in the earlier paper cited, where superparasitism is considered to produce merely a loss in efficiency.

If the reproductive rate of the parasite is > 1, then it will increase from generation to generation, in spite of the mortality by superparasitism.

In order to obtain a more accurate idea of the effect of the variation of the different factors considered, a few numerical examples may be taken.

We can show to begin with that the extermination of the host will occur soon after the value of μ reaches 1.

Let $\alpha = 1$. Our equation (10) becomes

$$\mu_t = e^{\mu_{t-1}} - 1.$$

Let $\mu_{t-1} = 1$. We have

$\mu_t = e - 1 = 1{\cdot}7182$, $\mu_{t+1} = e^{1{\cdot}718} - 1 = 4{\cdot}47$, $\mu_{t+2} = 89$, approximately.

Thus in three generations after the value of μ reaches 1, the host is exterminated. One more generation is required than where there is no mortality through superparasitism.

Suppose we have $\mu = \frac{ps}{nh} = \frac{100}{1000}$ and $\alpha = 1$, as before, we obtain from our equations the following values:

t	μ	H	P
1	0·100	904	96
2	0·105	814	90
3	0·110	729	85
4	0·116	649	80
5	0·123	573	76
6	0·131	502	71
7	0·140	436	66
8	0·150	375	61
9	0·161	320	55
10	0·173	269	51
11	0·189	223	46
12	0·208	181	42
13	0·231	143	38
14	0·260	110	32
15	0·296	82	28
16	0·345	58	24
17	0·411	38	19
18	0·508	23	14
19	0·624	12	11
20	0·866	5	7
21	1·377	1·3	3·7
22	2·93	0·06	1·24
23	17·71	—	—

The value of μ rises slowly during a considerable period and then more and more rapidly; the value of H falls at first rapidly and then more and more slowly; the value of P falls quite regularly, its curve being approximately a straight line.

If there were no superparasitism, the host would be exterminated in 10 generations; if the superparasitism caused only a loss in efficiency, but no mortality, the host would be exterminated in 13 generations (Thompson, 1924, p. 84); since it causes both mortality and a loss in efficiency, 23 generations are required.

Suppose now that the reproductive rate of the host is = 2, though still merely equal to that of the parasite. In that case the values of H and P are as follows (the values of μ being the same as in the preceding example):

t	H	P	t	H	P
1	1,805	195	13	1,181,050	307,074
2	3,252	358	14	1,822,608	539,492
3	5,827	677	15	2,710,197	935,019
4	10,377	1,277	16	3,841,526	1,578,868
5	18,350	2,404	17	5,094,862	2,588,190
6	32,193	4,557	18	6,274,399	3,915,325
7	55,987	8,399	19	6,724,972	5,823,826
8	96,446	15,528	20	5,658,373	7,791,571
9	164,443	28,449	21	2,879,579	8,337,167
10	276,615	52,268	22	325,192	5,433,966
11	457,971	95,259	23	<1	—
12	744,062	171,880			

Let the number of generations required for the extermination of the host by the parasite when there is (1) no superparasitism, (2) superparasitism with a loss in efficiency but without mortality, (3) superparasitism with both loss in efficiency and mortality, be designated respectively by t_1, t_2 and t_3.

Suppose that we have the reproductive rates of parasite and host equal and the ratio n/p equal to 20, 30 and 50, respectively. We have $\mu = 0{\cdot}05$, $0{\cdot}03$ and $0{\cdot}02$. We then obtain the following values for t_1, t_2 and t_3:

μ	0·05	0·03	0·02
t_1	20	30	50
t_2	23	33	53
t_3	42	75	134

Suppose we have $\alpha = 1{\cdot}5$. Putting $n/p = 10$, 100 and 1000, we obtain the following results:

μ	0·15	0·015	0·0015
t_1	4	9	15
t_2	4	10	16
t_3	6	11	17

Putting $n/p = 1000$ and $\alpha = 1{\cdot}5$, 3 and 5, we obtain:

μ	0·0015	0·003	0·005
t_1	15	6	5
t_2	16	7	5
t_3	17	8	6

Putting $\alpha = 5$ and $n/p = 1000$ and 10,000, we have:

μ	0·005	0·0005
t_1	5	7
t_2	5	7
t_3	6	7

Putting $n/p = 10{,}000$ and $\alpha = 2$, 5 and 10, we have:

μ	0·0002	0·0005	0·001
t_1	13	5	4
t_2	14	7	5
t_3	14	7	5

An examination of these examples makes the effect of the type of superparasitism here considered fairly clear.

When the reproductive power of the parasite is decidedly greater than that of the host, the effect is slight and may be allowed for by adding a generation or two to the figure obtained for t from the formula (5). An increase in the initial value of the host population as compared with the parasite population does not produce a proportionate increase in the number of generations necessary for control. The greater the superiority of the reproductive rate of the parasite as compared to that of the host, the less an increase in the host population as compared with the parasite population affects the final result. When the difference between the reproductive rate of the parasite and the host decreases, the retarding effect of superparasitism becomes more marked. Thus, if we have $\alpha = 1{\cdot}125$ (*i.e.*, the reproductive rate of the parasite only slightly superior to that of the host), control in a case where we have ten hosts to one parasite will occur in about seven generations if there is no superparasitism, whereas eleven generations will be required if there is superparasitism of the type we are discussing. On the whole, however, when the reproductive rate of the parasite is higher than that of the host only a slight retardation of the process of control occurs, as compared with cases in which there is no superparasitism or superparasitism causing no mortality.

When the reproductive rates of host and parasite are equal, the effect of the type of superparasitism here considered is much more serious. Taking the ratio of hosts to parasites as 20, 30 and 50 to 1, we find that the number of generations required for control is 2·1, 2·5 and 2·7 times the number required when there is no superparasitism. If circumstances were so unfavourable that the average female of both host and parasite were able to produce only one viable offspring in each generation, the mortality due to superparasitism would gradually eliminate the parasite, which would however, at least theoretically, eliminate its host before disappearing.

However, the cases in which the effective reproductive rate of the parasite is merely equal or very slightly superior to that of its host are of little interest to the entomologist attempting the colonisation of beneficial species in areas in which they have not hitherto existed, because such species cannot overtake the host in a reasonable time unless their introduced population is a much

larger one than it is ordinarily possible to obtain. The effect of superparasitism in such cases is thus of secondary importance.

SUMMARY.

It has been shown in an earlier paper that if the eggs of parasites are not deposited in unparasitised hosts alone, but distributed according to the laws of chance among unparasitised hosts and hosts already attacked by individuals of the same species, and if all the parasites develop successfully, no matter how many enter a single host, then the effect upon the time required for the extermination of the host, as compared to that which is required if only unparasitised hosts are chosen, is relatively slight.

The hypothesis examined in this paper is that when several parasites enter a single host, one, and only one, will develop successfully. It is shown that in such a case, provided the reproductive rate is decidedly greater than that of the host (*e.g.* 1·5 times as great), the effect on the time required for the extermination of the host by the parasite will be very little if at all greater than when superparasitism causes merely a loss in efficiency, as in the hypothesis previously examined. When the reproductive rate of the host is practically or actually equal to that of the parasite, the process of control will be seriously retarded, the delay being very much greater than in the preceding hypothesis. Control will, however, occur, even in this case.

Superparasitism of the type here examined will therefore not necessarily produce any marked retardation of the process of control in cases where control within reasonable time would otherwise be probable.

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ON THE STRUCTURE AND LIFE-HISTORIES OF BRITISH FRESH-WATER LARVAL TREMATODES.

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(With 5 Text-figures.)

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I. INTRODUCTION.

The fresh-water larval Trematode fauna of Great Britain is as yet very imperfectly known. The only early work of note is that of Thomas (1883) on the life-history of the liver fluke *Fasciola hepatica* L. From that time until 1923 there seems to be no published record of work on British fresh-water larval Trematodes. In that year Hesse (1923) described two cercariae from *Limnaea peregra* Müll., in Scotland, but their final hosts are unknown. In 1923 also, Vevers (1923) fed encysted stages of what he believed to be *Cercaria echinata* v. Sieb. from *Limnaea peregra*, to ducks, and obtained adults of *Echinostomum revolutum*. Three years later Brown (1926) described nine species, seven of which he claims as new to science, which parasitise the molluscs *Limnaea peregra* Müll. and *L. stagnalis* L. in the neighbourhood of Birmingham and Leeds. That author made some attempts to establish life-histories but these were not based on definite animal experiment.

From the Continent on the other hand there is a considerable record of fresh-water larval Trematodes, due chiefly to the labours of Nitzsch (1816), Ercolani (1881), von Siebold (1854), de Filippi (1854, 55, 57) and von La Valette St George (1855).

In America much faunistic work has been done of late on fresh-water larval Trematodes, the outstanding workers being Cort and Faust.

The larval Trematodes of South Africa have been described chiefly by Cawston and Porter, while the Indian forms have been investigated by Sewell.

During 1923–6 I engaged in a systematic search of the fresh-water Invertebrates of the Dundee and Arbroath districts for larval helminth parasites, and the present paper contains descriptions of six larval Trematodes obtained, together with the results of infection experiments I was able to carry out with certain of these parasites.

The work was carried out in the Natural History Department, University College, Dundee, under the late Prof. Gemmill, F.R.S., during my tenure of a Research Maintenance Allowance from the Department of Scientific and Industrial Research (1923–5), and a Carnegie Research Scholarship (1925–6).

A further account of these investigations will be published later.

II. MATERIAL AND METHODS.

The material investigated was collected in the Dundee and Arbroath districts, and from North Fifeshire in the neighbourhood of Newport and Tayport. Collections of fresh-water Invertebrates, tow-nettings and deposit examination from ponds and streams were made at different times during the year. The material was brought into the laboratory and examined alive there. In this way I have been able to get some idea of the incidence of infection in the various localities visited.

In the examination of the mollusca for parasites the usual method was to remove the shell and examine the tissues under a binocular microscope, teased either in water or 0·5 per cent. salt solution. In some cases where the larval Trematode infection had reached the stage for the discharge of cercariae, microscopical examination of the latter could be carried out immediately and the mollusc thus kept alive for future work. Measurements of the parasites were in all cases made from the living objects. Most of the structural points were also observed in the living specimens.

As regards anatomical details, those of the excretory systems of the cercariae were most difficult to make out. In this connection the best results were obtained from high-power studies of an abundance of living material. Thin cover-glasses were used, and by slowly removing water from the preparations the movements of the larvae were slowed down until observation under a $\frac{1}{12}$ oil immersion lens was possible. A Reichert stereo-inset in place of the ordinary ocular was found helpful in these observations.

The fixatives used were 5 per cent. formalin for parasites, and 10 per cent. formalin and dilute corrosive sublimate for hosts. For staining whole mounts I have found that borax-carmine (alcoholic or aqueous) gives on the whole good results, while for sections, haemalum followed by eosin gave uniformly good stains. Canada balsam and acetone balsam were used as enclosing media. The acetone balsam method (Ritchie, 1918) was suggested to me by the late Prof. Gemmill, and was found particularly useful in preventing shrinkage. Material for future reference was mostly preserved in 10 per cent. formalin.

III. EXPERIMENTAL INFECTION.

A. *Of Invertebrates.* I was able at different times to carry out infection experiments relating chiefly to the encystment of cercariae within the bodies of secondary intermediate hosts.

B. *Of Vertebrates.* With a view to the further elucidation of life-histories, I conducted, during 1926, a series of controlled feeding experiments using ducklings as final hosts. To the latter were fed encysted cercariae, and, after suitable periods the birds were killed under anaesthesia and the intestine and associated glands examined for adult parasites. These experiments were carried out under a vivisection licence from the Home Office. Details of the experiments follow the descriptions of the parasites to which they relate.

IV. SYSTEMATIC.

Classification.

The Trematode larvae described in this paper are arranged according to the classification proposed by Lühe (1909, pp. 175, 181). By this method the cercariae are arranged in groups based mainly on the character of the tail; that is, the classification is for the larvae only and is quite independent of life-history. Various workers have realised the artificiality of Lühe's classification.

Lebour (1912), for instance, in her review of the British marine cercariae, considers that in a primary classification the tail is not of so great importance as whether the cercariae develop in sporocysts or rediae and accordingly makes two primary groups:

(1) Developing in sporocysts.

(2) Developing in rediae.

Under the sub-divisions of these groups she indicates the type of final host (fish, bird or mammal). The author thus aims at a classification based on life-history—obviously the most desirable method. Unfortunately, however, relations between larvae and adults of fresh-water forms, based on complete experimental proof, are very imperfectly known.

Next to life-history as a basis of classification, Faust (1924) considers that a study of those systems of structure which are least modified in the course of development are of most use. He finds that there is only one common system carried over from larva to adult, namely the excretory system, and concludes, "data are now available to initiate the change (*i.e.* in classification) almost wholly on the basis of excretory system."

Accurate tracing of the excretory systems of cercariae, however, is in all cases exceedingly difficult, even with suitable apparatus and an abundance of living material. In the descriptions of practically all European species, including those of such recent workers as Szidat (1924 *a*, *b*) and Wunder (1923 *a*, *b*, *c*), details of capillary and flame-cell structure are lacking. Brown (1926), in this country, has been able, in most cases, to observe the finer details in the species described by him.

It is hoped that the details given in this paper will provide a correct and recognisable description of a few of these forms, based on personal observations. This procedure I consider very desirable since it is only by such methods that gradually a complete revision of this difficult group will be attained.

Identification.

On account of the inadequate descriptions of the great majority of European forms, the identification of cercariae is an exceedingly difficult task. Through recent investigation of a comparatively small number of forms, advances in our knowledge of minute structure, and to a less extent of life-history have resulted, but the majority have still to be examined in the light of modern knowledge and methods. In my own case, I have fortunately been able, by means of direct animal experiment, to refer two of the six larvae described to known adults. Of these two, one is doubtfully *Cercaria monostomi* v. Linst., while the other is presumably identical with an unnamed form briefly described by Mathias (1926) as the larva of *Echinoparyphium recurvatum* v. Linst. While the four xiphidiocercariae described appear to be new I have also compared them with what appear to be their nearest European relatives, and pending further evidence as to their life-histories, I have referred to them as *Cercaria* X. 1, X. 2, X. 3 and X. 4, respectively.

V. DESCRIPTIONS OF PARASITES.

Monostome cercariae (Lühe, 1909, pp. 177–80).

The life-history of Notocotylus seineti *Fuhrmann.* (Fig. 1.)

From this area I have recorded one trioculate monostome larva. Its adult, as shown by infection experiment, is *Notocotylus seineti* (Fuhrmann).

The life-cycle of this Trematode resembles that obtaining in the *Monostoma* generally. That is, development takes place in rediae, while the encysted stage in known instances is free on solid objects, such as the shells of molluscs and waterweeds. The cercariae have only one sucker, which is situated anteriorly round the mouth. All have a pair of pigment eye-spots on the anterior dorsal surface of the body, and some have in addition a median pigment spot slightly anterior to these.

Lühe refers *Cercaria imbricata* Lss and *Cercaria ephemera* Nitzsch to probable adults, but experimental confirmation is lacking.

The rediae occur in the liver and gonads of *Limnaea peregra* Müll. and *Physa fontinalis* L. They are dark brown cylindrical structures tapering slightly towards each end. The length at maturity varies from 3 to 4 mm. and the breadth from 0·4 to 0·5 mm. All stages in development may occur in the liver of a host, but mature rediae were only met with near the surface of the gland. The very young rediae occur deep in the substance of the liver and are colourless, and almost motionless. As growth proceeds they acquire the power of elongating and contracting slowly. Later a side to side movement is evident, becoming more pronounced towards maturity. Aided thus, the

rediae appear to migrate slowly towards the surface of the digestive gland where they acquire the characteristic shape and colour.

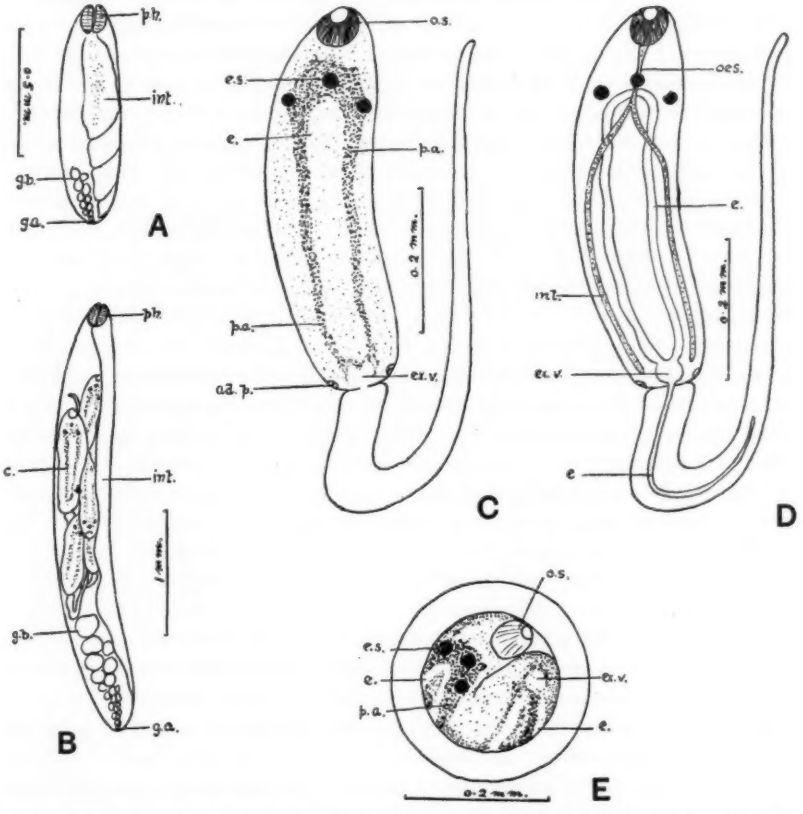

Fig. 1. Larval stages of *Notocotylus seineti* Fuhrmann. *A*. Young redia showing septate intestine and developing cercariae. *B*. Mature redia. *C*. Cercaria showing pigmentation. *D*. Cercaria showing alimentary canal and excretory system. *E*. Encysted cercaria.

LETTERING FOR FIGURES 1—5.

a.p. ambulatory process; *ad.p.* adhesive pocket; *b.p.* birth pore; *c.* cercaria; *c.c.* ciliated canal of excretory system; *co.* collar; *c.s.* cirrus sac; *e.* excretory vessel; *e.s.* eye-spot; *ex.p.* excretory pore; *ex.v.* excretory vesicle; *f.c.* flame cell; *g.* rudiment of genital system; *g.a.* germinal area; *g.b.* germ ball; *int.* intestine; *n.* nervous system; *oes.* oesophagus; *o.s.* oral sucker; *ov.* ovary; *p.a.* pigment area; *ph.* pharynx; *p.ph.* prepharynx; *s.g.* salivary gland cell; *st.* stylet; *t.* testes; *v.s.* ventral sucker.

Development is in some instances accompanied by transverse constrictions in one or more places. This feature is no doubt due to varying resistance of the host tissues and not to incipient transverse fission. In all heavy infections the spire of the host, between the shell and the body wall, becomes packed

with mature rediae which issue singly when the mollusc is opened. The liver and gonads are usually quite destroyed.

Internal anatomy. Young rediae measuring 0·3 to 1·0 mm. in length have a distinct pharynx and a relatively wide intestine reaching almost to the posterior end of the body. One or more oblique transverse septa are present in the distal portion of the intestine. In older forms, 2 to 4 mm. in length, the intestine is non-septate, and is usually less conspicuous owing to its becoming pressed to one side by developing cercariae. The intestines of the older rediae appear sometimes more, and at other times less filled with food material derived from the liver. In the middle of the body the wall is 0·01 mm. in thickness and there is an increase to 0·05 mm. at the posterior pole. The region around the pharynx is solid. The cuticle is thin but tough, and contains numerous highly refractive granules. Internal to the cuticle there is a single layer of flattened cells. At the tail end of the redia there is a conical mass of germ cells proliferated as a central rachis from a small area of epithelium at the extreme posterior pole. This mass is connected to the walls by fine strands. In it, the germ cells become enlarged, and by repeatedly dividing produce the cell masses which give rise to the cercariae. The latter remain for a time attached to the walls by fine strands, but later, and before maturity is reached, become free in the cavity of the redia. From eight to twelve mature cercariae may occur in one redia. No birth pore was observed.

The cercaria is of a dark brown colour and almost opaque. The pigmentation is strongest along two dorsal and two ventral lines running the whole length of the body. Normally while in suspension in water the length is 0·55 to 0·6 mm. with a maximum breadth of 0·18 mm. near the centre of the body. The larva is exceedingly muscular and is capable of stretching to almost twice the above length. It is smooth, and slightly convex dorsally and concave ventrally, the concavity being more pronounced posteriorly. The posterior end is definitely rounded and contains two dorso-lateral adhesive pockets. The tail is stout and muscular and as long as or slightly longer than the body. There is one sucker situated anteriorly round the mouth.

Prominent in the anterior dorsal region of the mature cercaria, are three black pigment eye-spots arranged in the form of a triangle. They develop from an irregularly pigmented area on the dorsal-anterior aspect of the body. The process is a gradual one, the cyclopean eye being formed later than the lateral pair. Occasionally the median eye is not formed at all, there being then two lateral eyes and between them scattered pigment granules. This condition is not uncommon even in the encysted stage of the cercaria.

For other measurements see Table I.

Internal anatomy. The oesophagus is short and narrows slightly towards its bifurcation just behind the median eye. In transverse section it is oval and thick-walled but shows nothing in the nature of a true pharynx. The intestinal caeca are relatively narrow and extend almost to the posterior end of the body.

The excretory vesicle is a clear sac at the hind end of the body. Two conspicuous lateral canals, united by a transverse duct a little below the level of the eyes, empty into the vesicle. A median duct connects posteriorly with the tail. The excretory pore is dorsal.

Cystogenous gland cells are numerous all over the body. The posterior locomotor pockets are lined on their inner faces with a group of 6–8 special gland cells which pour out a secretion for the attachment of these organs to the surfaces of contact.

Table I.

	mm.
Oral sucker	0·05
Length of tail	0·5–0·65
Breadth of tail at base	0·056
Excretory vesicle	0·02
Diameter of eye-spots	0·01
Distance apart of lateral eyes	0·07
Distance of median eye from anterior end	0·06

The encysted stage. The cercaria encysts on the shells of the same host species. The cysts occur chiefly round the mouths of the shells on the outer surface, none were found near the apices. Limnaeas already infected with rediae rarely bear encysted stages on their shells. This phenomenon seems to be due to the fact that the presence of rediae in the digestive gland of the mollusc greatly diminishes the amount of mucus normally present on the shell, and the mucus if not actually necessary for encystment, greatly facilitates its process. The cyst is hemispherical, its flattened base being in contact with the shell of the host. The diameter is 0·24 mm. including the wall which is 0·02 mm. thick. The latter is fairly transparent and through it can be made out the eye-spots and excretory system of the larva.

In nature the encysted stages occur to a less extent on the shells of the following molluscs: *Limnaea truncatula* Müll., *Valvata piscinalis* Müll., *Planorbis albus* Müll., and *Physa fontinalis* L. On one occasion, two cysts were found on the valve of the ostracod *Eurycypris pubera*.

Occurrence. Observations on material made at all seasons during 1925 gave the following results: the first infection was noted on February 20th when immature rediae occurred in one out of ten Limnaeas examined. Mature rediae and active cercariae were present in 8–10 per cent. of the specimens examined during March and April. The infection appeared to reach its maximum about the end of May or the beginning of June. During these months 15–20 per cent. of the molluscs dissected contained mature rediae. Limnaeas opened during July and August contained many empty rediae, and relatively few active cercariae. The parasite occurred in less than 5 per cent. of the specimens of *Physa fontinalis* L. examined during June and July 1925.

The encysted stages occurred most abundantly during the summer months but they were also met with in the winter, long after motile cercariae had disappeared. During June and July about 60 per cent. of the Limnaeas were infected, each infected specimen carrying from 3–20 encysted cercariae.

Experimental.

A. The cercariae swim actively head first with the body slightly elongated and directed forwards and downwards. When active movement ceases they sink slowly to the bottom of the dish, coming to rest on the illuminated side with the body symmetrically placed towards the light. Tailless larvae are able to progress in a leech-like manner by means of their posterior adhesive pockets. Non-encysted cercariae survive in pond water for 15 to 20 hours.

Proof that the cercariae encyst on the shell of the same host species was obtained experimentally by introducing two heavily infected specimens of *L. peregra* Müll., together with 20 young Limnaeas of the same species, into a glass tank containing pond water. A superficial examination of the shells of the young Limnaeas made before the experiment revealed no encysted stages. Control molluscs kept in a separate tank, free from infection, were also carefully examined. Observations were made every other day as long as the material lasted.

Summary of results:

(1) Encysted cercariae were found on 14 of the young Limnaeas in contact with the infected species. Encystment in all cases had taken place round the mouths of the shells; no cysts were found on the spires.

(2) Approximately one-fourth of the cercariae liberated had encysted.

(3) No encysted stages were found on the shells of the two rediae-infected Limnaeas.

(4) Three encysted stages were found on the bottom of the glass tank.

(5) Control molluscs all negative.

In the process of encystment, cystogenous fluid is first secreted apparently from the entire dorsal surface of the larva. The body then contracts appreciably, the anterior end broadening and gradually becoming bent under the more concave posterior end. Uniformity of the cyst wall is ensured by slow half-rotations of the larva. The tail usually separates soon after encystment has begun. The finished cyst is hemispherical, the wall consisting of two distinct layers, an exceedingly resistant inner layer and a gelatinous and somewhat more transparent outer layer.

B. A fairly plentiful supply of encysted stages being available, I conducted, during April 1926, controlled feeding experiments using ducklings as final hosts. As a result a number of the corresponding adults, together with a series of intermediate forms from the encysted stage onwards, were obtained. The parasite was located in the rectum and caeca and was identified as *Notocotylus seineti* (Fuhrmann, 1919). This monostome has been previously recorded from the Teal (*Querquedula querquedula*) but its life-history was previously unknown.

Identification of cercaria.

The only European species comparable with the larva of *Notocotylus seineti* is *Cercaria monostomi* v. Linst. from *Limnaea peregra* Müll. and *L. ovata* Drap.

For detailed comparison, however, the original description of *C. monostomi* (v. Linst. 1897, pp. 76–7, figs. 4–7) is inadequate. The posterior end is described as rounded, but there is no record of adhesive pockets in this region. The latter, in my own species, are well seen in the living specimens but tend to disappear almost completely when the animal is fixed.

Recently, Wunder (1923 *a*) has described, in more detail, a cercaria from *Limnaea ovata* Drap. which he considers identical with *C. monostomi* v. Linst. Table II gives a brief comparison of the three larvae in question, and it will be seen that the cercaria of *Notocotylus seineti* Furhm. is in close agreement with that of Wunder, and may possibly be identical with *C. monostomi* v. Linst.

Wunder deals more particularly with the structure, development and function of the tail, and gives no account of the redia stage. Encystment is described in detail, the main conclusions of the author being that in the process the body integument swells up, beginning at the anterior end and that a secretion from the oral sucker also helps in the formation of the cyst wall. He has observed this swelling of the body integument in the free swimming state before a place for encystment is reached. The encysted stages occur on the leaves of *Elodea canadensis* and *Myriophyllum*, and on the shells of young specimens of *Limnaea ovata* Drap. and *Planorbis marginalis* Drap.

Table II.

C. monostomi v. Linst.	*C. monostomi* (Wunder)	Cercaria of *Notocotylus seineti* Fuhr.
Rediae		
Lengths: up to 2·0 mm.	No record	Up to 4·0 mm.
Cercariae		
Body: 0·62 × 0·2 mm.	0·45–0·69 × 0·015 mm.	0·55–0·60 × 0·16 mm.
Tail: 0·48 mm. shorter than body	0·3–0·52 mm.	0·5–0·65 mm. as long as or slightly longer than body
Diameter of eye-spots: 0·0052 mm.	No record	0·01 mm.
Small pharynx behind oral sucker; course of intestine unknown	Oesophagus without conspicuous pharynx; loops of intestine reaching to posterior end of body	As in *C. monostomi* (Wunder)

ECHINOSTOME CERCARIAE (Lühe, 1909, pp. 187–8).

The life-history of Echinoparyphium recurvatum *v. Linst.* (Fig. 2.)

The rediae were found in the liver of *Valvata piscinalis* Müll. When mature they are inert pale yellow bodies varying in length from 1·0 to 1·5 mm. and in breadth from 0·18 to 0·22 mm. The head region is more transparent than the rest of the body and is separated from it by a slight constriction. Anteriorly there is a prominent pharynx, 0·11 mm. in diameter, and a thick-lipped birth pore which is capable of protrusion and withdrawal. The cuticle is thick, and between collar and mouth is often marked by numerous transverse constrictions. The young rediae are colourless and transparent. They possess a muscular oral pharynx and a prominent collar anteriorly, and a pair of

mobile lateral amulatory processes near the posterior end. These processes completely disappear at intervals when the tail end of the redia becomes pointed. The older rediae lose the lateral appendages almost completely but the collar remains distinct.

Internal anatomy. The smallest rediae observed were approximately 0·3 mm. long by 0·06 mm. broad, and had a muscular pharynx leading to an empty sacculate intestine reaching a little below the level of the collar. These rediae contained no developing cercariae. Larger specimens measuring 0·5 mm. in length contained them, the intestine being still of the same relative length

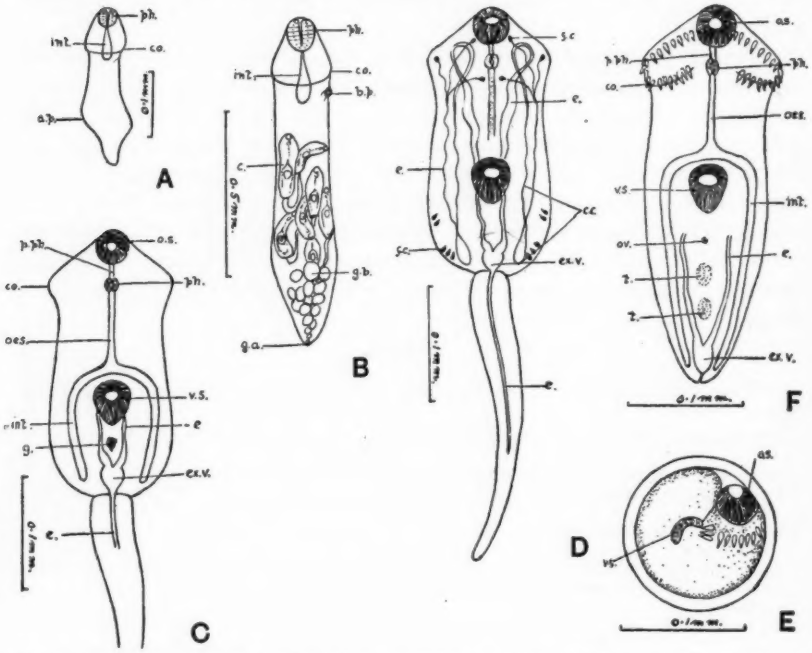

Fig. 2. Larval stages of *Echinoparyphium recurvatum* v. Linst. *A*. Young redia. *B*. Mature redia. *C*. Cercaria showing alimentary canal. *D*. Cercaria showing excretory system. *E*. Encysted cercaria. *F*. Larva removed from cyst. (For lettering see Fig. 1.)

but now containing food particles. The germinal area from which the cercariae arise is situated in the posterior region of the redia. The birth pore was not in rediae less than 0·7 mm. long. Rediae 0·5–1·0 mm. in length exhibit most movement and in these longitudinal and transverse striations can be made out on the cuticle. The mature redia is motionless and its intestine though short is still conspicuous.

The cercaria is small and oblong ovate in contour. The length is about 0·25 mm. and the breadth 0·12 mm. near the posterior end. An anterior constriction forms a prominent collar round which there is an incomplete

circle of 43 to 45 spines arranged regularly and symmetrically in a double row. The spines in the oral row are very slightly shorter than those in the aboral row. Behind the collar the body is covered with smaller spines dwindling towards the ventral sucker. There are two suckers; a pronounced terminal oral sucker with a deep cavity, and a somewhat larger ventral sucker situated in the posterior third of the body. The tail is blunt, stout, and muscular, and about the same length as the body. The caudal pocket is terminal.

Further measurements are given in Table III.

Internal anatomy. The dilated oral cavity becomes constricted as it reaches the region of the pharynx. The latter organ is muscular and prominent and lies in the plane of the collar. A long narrow oesophagus forks a short distance in front of the ventral sucker. The intestinal caeca extend to almost the posterior end of the body.

Excretory system. The conspicuous elements consist of a spherical excretory vesicle, two main lateral collecting tubules, and a single tubule in the tail. The main collecting tubules of the body become constricted in the region of the collar and there becomes bent inwards and downwards. The secondary tubules so formed can be traced backwards to the posterior end of the body where each loops forward and ends in a flame cell situated at the lower margin of the oral sucker. Slightly below the level of the pharynx two capillaries with smaller flame cells enter the tubule. Five additional flame cells were observed on either side in the posterior portion of the trunk but their capillaries were not traced. The lower portions of the secondary excretory tubules are ciliated. Similar ciliated areas have been observed in *Cercaria coronata* Fil. from *Limnaea stagnalis* L. and *L. palustris* Müll. and in *Cercaria limbifera* from *L. palustris* var. *corvus* (Seifert, 1926). In the tail the system is represented by a single median tubule extending about three-quarters of its length and during that distance it remains unbranched.

Two glandular masses consisting of numerous cells occupy a large area a little anterior to the centre of the body. They lie ventral to the excretory system and open by numerous fine lateral ducts into the oral cavity.

Genital system. This is represented by a single mass of small round cells about midway between the ventral sucker and the posterior extremity of the body.

Table III.

	mm.
Oral sucker	0·035
Ventral sucker	0·04
Length of tail	0·25
Breadth of tail at base	0·035
Collar spines: oral	0·01
aboral	0·012
Pharynx	0·014
Excretory vesicle	0·02

The encysted stage. The cercaria encysts in the same host species and the cysts, of which there may be fifty or more present, are located chiefly in the

liver and tissues of the mantle. The encysted stages also occurred in about 20 per cent. of *Valvata piscinalis* Müll. and *Planorbis albus* Müll. during the summer of 1925, from 3 to 10 cysts being present in each infected specimen. The cyst is spherical and transparent, and the larva within is practically quiescent. The diameter is 0·11 to 0·13 mm. excluding the wall which is 0·015 mm. thick. Apart from the genital system, the larva when removed shows very little in the way of further development. Two oblong ovate testes are now observable, one behind the other in the posterior region of the body. The ovary is spherical and lies in the median line near the posterior margin of the ventral sucker. Forty-five collar spines can now be definitely made out. Those in the oral row are approximately 0·013 mm. long and those of the aboral row 0·015 mm. Four or five spines near the mid-ventral line, on either side, are approximately 0·02 mm. long.

Experimental.

A. The cercariae swim actively by lashing movements of their tails. In tap water all movement ceases in less than six hours. In nature it seems probable that all the cercariae escape via the birth pore, and that encystment normally takes place in the host harbouring the rediae. That the cercariae are able to migrate from the first host, however, is proved by the occurrence of encysted stages in molluscs free from rediae.

B. During May and June 1926 I fed encysted stages to ducklings. As a result, adults of *Echinoparyphium recurvatum* v. Linst. 1873, were obtained from the large intestine. Six ducklings were used and examined at intervals, so that a number of intermediate stages of the parasite were also obtained. Infection takes place readily and development is normal. Eggs were found in the faeces 18 days after infection. *E. recurvatum* occurs naturally in *Nyroca marila* L., *N. fuligula* L. and *Gallus domesticus* L. Dietz (1910) says nothing of its life-history.

Identification.

There is no named fresh-water cercaria comparable with the larva of *Echinoparyphium recurvatum* v. Linst., but since the above experimental work was performed, Mathias (1926) has fed encysted stages of an Echinostome larva from *Planorbis planorbis* L. to ducks and obtained *E. recurvatum* v. Linst. The larva used by that author in his experiments must therefore be identical with that described above, but as he has given no recognisable description of the redia or cercaria stages these are described here in detail for the first time.

XIPHIDIOCERCARIAE (Lühe, 1909, pp. 189–200).

These forms called xiphidiocercariae by Lühe are numerous, and although perhaps not closely related their assemblage in a single group is convenient. They are, as a rule, small, slender tailed cercariae with a rounded anterior margin armed with a boring spine or stylet, usually in the roof of the oral

sucker. The length of this stylet may vary a little in the same species, but its form appears to be very definite in each species, and varies in different species distinctly enough to serve, in many cases, as a mark for diagnosis. Eye-spots are usually wanting. Development in sporocysts is most frequent, and encystment in a secondary intermediate host usual, in cases where the further life-history is known.

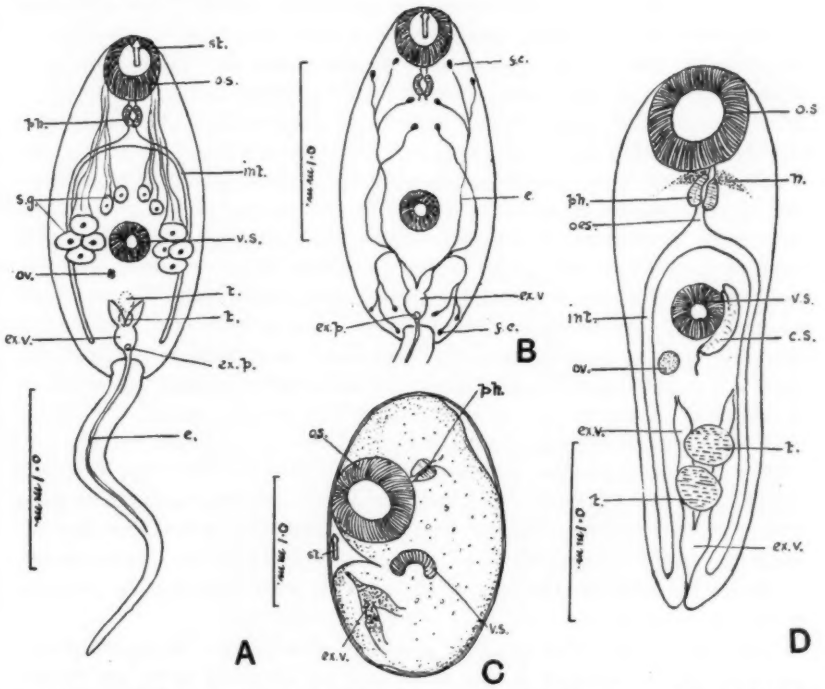

Fig. 3. *Cercaria* X. 1. *A*. Cercaria showing alimentary canal and glands. *B*. Cercaria showing excretory system. *C*. Encysted cercaria from *Gammarus pulex*. *D*. Larva removed from cyst. (For lettering see Fig. 1.)

Cercaria "X. 1." (Fig. 3.)

The sporocysts occur in the liver and gonads of *Limnaea peregra* Müll., and when mature are blunt, short, semi-transparent sacs. In all the body narrows slightly towards both poles. Young sporocysts are colourless but they gradually assume an orange tint which in heavy infections imparts a sickly yellow colour to the shell of the host. Mature sporocysts measure from 1 to 2·5 mm. in length and from 0·3 to 0·45 mm. in breadth. There is no movement apart from that caused by the active cercariae within.

The wall is 0·014 mm. thick and made up of two layers of cells. The external layer is composed of flattened cells and contains in addition to the orange pigment scattered granules of black pigment. The internal epithelial

layer is best seen in young sporocysts when the early embryos are still attached to it. The area of cell proliferation is non-localised lining the whole cavity of the sporocyst.

In all Limnaeas examined, the sporocysts were found arising within the digestive gland, which organ they finally completely riddle. Usually also, the gonads become involved and are quite destroyed, but in no case were they found to be the starting point of infection. Development is in most cases accompanied by transverse constrictions on the sporocysts and eventually they become much bent upon themselves. A certain amount of transverse fission seems to take place also, especially in the younger sporocysts. In heavy infections the sporocysts become exceedingly involved and it is impossible to obtain an undamaged one owing to fusion of their walls.

The cercaria is semi-transparent and very contractile. The normal form, while in suspension in water, is oral with the maximum breadth slightly anterior to the centre of the body. The cuticle shows superficially, in its anterior two-thirds, fine parallel lines in which spines are not definitely recognisable. The body is convex dorsally and concave ventrally, the concavity being more pronounced in the posterior and less contractile half. Around the dorsal, lateral and part of the ventral aspects of the extreme posterior region there is a rounded rim-like thickening. This thickening is less pronounced on the ventral margin of the caudal pocket which is sub-terminal. The tail is muscular and rather longer than the body. There are two suckers. The oral sucker, which is the larger, is armed with a stylet in its dorsal lip. The total length of the stylet is 0·026–0·029 mm., the average measurements of its parts being: shaft 0·022 mm. long by 0·003 mm. broad, nib 0·006 mm. The nib bears two minute and slightly downwardly directed blade-like reinforcements on its lateral surfaces. The ventral sucker is situated very slightly posterior to the centre of the body and may be slightly protruded.

Internal anatomy. The mouth leads almost directly to a distinct muscular pharynx. The oesophagus is very short and the intestinal caeca are narrow and extend to within a short distance of the posterior end of the body. The caeca are slightly dorsal in position and in the majority of cercariae examined were only recognised as two narrow lines of refractive cells arranged end to end.

The excretory vesicle is in the form of a **Y**. The posterior region is contractile and connected by a narrow neck to the two non-contractile arms. The length of the median stem is about 0·02 mm. and in the contracted state it becomes wedge-shaped, while the lateral arms become more closely opposed, and the "neck" almost invisible. The excretory pore is dorsal. Each arm of the excretory vesicle receives a main lateral excretory duct which can be traced anteriorly to the lower margin of the oral sucker where it ends in three flame cells. A second excretory tubule arises as a fairly large flame cell slightly posterior to the bifurcation of the oesophagus and follows a course almost parallel to the intestinal loop, joining the main duct just before the latter enters the arm of the excretory vesicle. A third, and less sinuous

tubule arising in three capillaries with flame cells in the extreme posterior region of the body, passes anteriorly, and opens into the main lateral duct near its junction with the first tributary. A median excretory duct from the tail opens into the posterior end of the vesicle.

The genital system, testes and ovary, is represented by as yet only feebly differentiated masses of cells. The testes are ventral in position and occur close together about mid-way between the ventral sucker and the posterior margin of the body. The ovary occurs dorsally near the posterior margin of the ventral sucker on the right side.

On either side of the body there are six large salivary gland cells arranged in two groups. The posterior group on each side consists of four closely opposed cells situated almost on a level with the ventral sucker. The cells of this group are transversely elongated and measure 0·02 by 0·014 mm. Anterior to the ventral sucker and inclined somewhat towards the median line are the other two groups made up of two cells each. The latter are rounded, their diameter being approximately 0·01 mm. All these gland cells have prominent nuclei, and the contents of the posterior group are finely granular. From the glands, ducts pass anteriorly to the oral sucker and open near the base of the stylet. Cystogenous gland cells with granular contents are present all over the body but are most abundant in the posterior two-thirds.

For other measurements see Table IV.

Table IV.

	mm.
Length of body	0·2–0·25
Breadth of body (maximum)	0·12
Breadth of body posteriorly	0·06
Thickness of body	0·025
Diameter of oral sucker	0·04
Diameter of ventral sucker	0·028
Diameter of pharynx	0·014
Length of tail	0·25

The encysted stage occurs in numerous fresh-water Invertebrates, chiefly *Gammarus pulex* L., and to a less extent in certain aquatic insect larvae. Owing to a slight variation in the size and shape of the cysts from the various hosts, it was at first thought that they might be of different kinds, but that this was a mistaken idea was shown by a detailed examination of their contents and by subsequent experimental work. In all natural infections the contents of several cysts were examined microscopically, special attention being paid to the relative sizes of the suckers, body armature, including stylet and the genital system. In nature the cysts occur most abundantly in *Gammarus pulex* and a description from this source may be regarded as typical.

The cysts are oval or rarely spherical in shape and vary more or less in size. A series of measurements show a range in length from 0·2 to 0·27 mm., and a breadth from 0·15 to 0·19 mm. The average length is about 0·23 mm., and the breadth 0·17 mm., all measurements being taken from uncompressed

cysts. The wall of the cyst is a transparent membrane about 0·002 mm. in thickness and through which the larva may be readily observed. The latter occupies almost all the space within the cyst, its most common position being with the anterior or posterior extremities, or both, bent ventrally. Through the wall can be seen the organs of the larva: oral and ventral suckers, alimentary tract, excretory vesicle, and such genital organs as are developed. Owing to movements of the body, however, identification of these organs is complicated.

The larva freed from the cyst is mobile. The most common form is an elongated oval, the posterior two-thirds being of nearly uniform breadth. The anterior end is rather more obtuse than the posterior. The length varies from 0·42 to 0·44 mm., and the breadth from 0·12 to 0·16 mm. The transverse markings on the body are now more distinct, particularly in the anterior third, and in addition transverse rows of minute spines can be detected in the anterior region as far back as the level of the ventral sucker. The oral sucker is large, spherical, and directed ventrally. The ventral sucker is much smaller than the oral sucker; it is also spherical and is situated near the centre of the body. The average diameter of the oral sucker is 0·09 mm., and of the ventral sucker 0·045 mm.

Internal anatomy. The prepharynx is short. The pharynx is muscular and measures 0·03 mm. long and 0·025 mm. broad. The oesophagus is very short and the intestinal caeca are prominent and extend almost to the posterior margin of the body.

The excretory vesicle has increased greatly in size but still retains its Y shape. The main disposition of the excretory tubules and their tributaries remains the same. Two additional flame cells were observed on each side in the posterior region of the larva.

Two testes arranged somewhat obliquely one behind the other occur about mid-way between the ventral sucker and the posterior margin. They are almost spherical, the diameter being approximately 0·04 mm. The vas deferens arises from the inner surface of each but its further course could not be traced. Just above the ventral sucker on the left side is a curved cirrus sac measuring 0·07 by 0·01 mm. but its connection with the other genital organs could not be determined. The ovary lies to the right of the median line, a little below the margin of the ventral sucker.

The nervous system is represented by a saddle shaped mass with two lateral ganglia lying across the prepharynx. Short lateral nerves were seen in a few.

A comparison of the above description with that of the cercaria when it emerges from its host shows:

(1) An increase in size of the body generally, this being very pronounced in the case of the oral sucker.

(2) That the alimentary, excretory, and genital systems have become more definite.

(3) The absence in the encysted stage of conspicuous glands associated with the oral sucker. These probably function only in process of penetration and encystment, and then disappear.

The encysted stages in *Gammarus pulex* are usually located in the tissues of the hepatopancreatic tubes or in the masses of connective adipose tissue ensheathing the alimentary canal and gonads, and also in the connective tissue separating the bands of muscle, and attached to the ventral nerve cord. On one occasion a transverse section through the body of the host showed an encysted stage about the middle of the basipodite of one of the pereipods.

Occurrence. When first studied, during June and July 1923, over 90 per cent. of the older Limnaeas from a stream near Arbroath were heavily infected. Observations on material from this locality during 1924 gave the following results: the first infection noted about the middle of February, two Limnaeas out of twenty examined being infected. The sporocysts occurred in the liver, the gonads being as yet unattacked. They were immature, colourless and not numerous. At the beginning of March five immature infections occurred in ten Limnaeas dissected. The cercariae were fairly active when removed from the sporocysts, but were found useless for infection experiments with secondary intermediate hosts. Sporocysts liberating cercariae were found in the field towards the end of March. The infection appeared to reach its height towards the end of June, when practically all the older Limnaeas were again heavily infected. Molluscs opened during August, September and October contained as a rule many empty sporocysts and comparatively few immature cercariae.

The encysted stages in *Gammarus* while occurring most abundantly in spring, summer and early autumn, *i.e.* during the period of active migration of cercariae from their molluscan host, may also be found all through the winter. During June and July 1924 over 80 per cent. of the *Gammarus*, young and old, were infected. Forty to fifty cysts were commonly met with, and on one occasion 132 were counted in a single host. Males and females are alike infected.

Experimental.

Emergence of cercariae from the host. Although I have not been able to follow the escape of cercariae from their snail host over any length of time, the following experiments performed in this connection are of interest.

Exp. 1. A *Limnaea* known to be liberating cercariae was kept in pond water in a small glass jar. Under these conditions, over a period of nine hours during the day, 381 cercariae were liberated, the emergence taking place in six swarms at irregular intervals. A count made next morning showed that during the night 103 cercariae had been liberated. The temperature of the water varied from 15 to 20° C.

Exp. 2. The *Limnaea* used in Exp. 1 was placed in a larger vessel the water being kept in constant but not rapid motion, by means of a mechanical

stirrer, from 9 a.m. till 4 p.m. At the end of the experiment 233 cercariae were recovered.

Exp. 3. Exp. 1 repeated with jar placed in a drying oven in diffused light. Temperature of water 29° C. Under these conditions 452 cercariae were liberated over a period of 9 hours.

Conclusions. (1) Cercariae escape during the whole 24 hours, but the numbers are greater during the day time. (2) Agitation of the water retards their escape. (3) Increase in temperature accelerates their escape.

The only detailed record of the escape of cercariae from their snail hosts is that of Cort (1922). In his experiments he used certain furcocercous and echinostome cercariae, and also an undetermined xiphidiocercaria. His results for the latter closely agree with my own, *e.g.*:

(1) "The stylet form studied escaped from its host during the whole 24 hours although there was a distinctly smaller number which escaped during the night."

(2) "The temperature of the environment very significantly affected the numbers of cercariae escaping...temperature only slightly below normal will inhibit their escape."

Cort thinks that further work along this line will be profitable. "It is very possible," he says, "that a study of this same problem for the cercariae of human Trematodes may shed light on the general problem of human infestation and give data of value in control."

Activity and duration of free life.

The cercariae swim actively head first towards the surface of the water. Short periods of activity are alternated by longer periods of rest during which the larva sinks slowly, with its tail projecting almost at right angles to the body. While in motion the tail is bent well under the body. Decaudation is not readily effected. Non-encysted cercariae may survive for 36 hours in pond water.

Mode of entrance of cercariae into Gammarus pulex.

Specimens of this crustacean for infection experiments were obtained from a locality where no helminth infection of this nature had previously been found. As a further control, a large number were examined before each experiment.

As regards mode of entrance and process of encystment in *Gammarus*, accurate observation is difficult owing to the activity of the host and the opacity of its cuticle. The first difficulty was in part overcome by pressing the head of the crustacean firmly between forceps, thereby checking all movement save that of the respiratory appendages. The movements of cercariae attaching themselves to the body of the host can then be followed.

The cercariae do not appear to exhibit any special activity in seeking out a host, but very active leech-like movements begin when one is reached. They enter both dorsally and ventrally at intersegmental regions where the body

integument is thinnest, and usually the middle intersegmental regions are selected for the purpose. The tail is shed before penetration is accomplished. I have not observed the actual process of encystment in *Gammarus*, but that it takes place very shortly after penetration is shown by the following: a *Gammarus* placed for five minutes in close contact with many active cercariae, provided on dissection twenty minutes later, 5 cysts from the tissues of the hepatopancreatic tubes, and 4 from the connective tissue just under the exoskeleton. All cysts were thin-walled and oval, and the larvae with stylets still *in situ* were actively rotating within.

Mode of entrance and process of encystment in Chironomus plumosus.

C. plumosus is one of the numerous insect larvae in which the cercaria encysts naturally and on account of its transparency readily lends itself to observations on the entry and process of encystment of the cercariae.

The entrance is usually effected dorsally at the last few intersegmental regions of the host's body. In the process the larva does not exert itself to any great extent. The cuticle of the host appears to be dissolved by a secretion from the stylet glands, but the stylet itself does not take a very active part in penetration. From 2 to 3 minutes usually elapse from the time the cercaria begins to penetrate until it is actively rotating under the host's cuticle in the process of forming a uniform cyst. Decaudation is usually effected before the cercaria penetrates, but on two occasions I have observed the tail still attached to the cercaria within the body cavity of the host. These were shed, however, before encystment commenced. Encystment begins almost immediately after penetration. Only very rarely do the cercariae move more than the length of one body segment from the point of entry, and one seldom finds a cyst anterior to the first of the last three segments of the body. The contents of the stylet glands are not completely used up in penetration, but on examination 2 or 3 hours later they can scarcely be recognised. It is probable, therefore, that these glands assist in the formation of the cyst wall as well as in the penetration of the host's cuticle. In the actual process of encystment, the secretion from the cystogenous cells covers the surface of the cercaria, and becomes evenly distributed by the undulating and rotating movements of the body. The stylet is shed before, or soon after, encystment and may be seen free within the cyst or more rarely embedded in its wall.

The rôle of the stylet glands of the xiphidiocercariae is not yet fully understood. The above observations are in agreement with those of Wunder (1923 *c*) for *Cercaria intermedia*. This author considers the stylet glands of chief importance in penetration, but that the stylet may also function in hard places. Hesse (1923) in his observations on the encystment of an undetermined xiphidiocercaria on the shell of *Limnaea peregra* Müll., states that the stylet glands probably co-operate in the formation of the cyst wall. *Cercaria intermedia* of Wunder encysts in a whole host of insect larvae, and among these the writer distinguishes "Corethra" and "Ephemerid" cysts by

the nature of the wall formed in these two types of host. In my own case, while there is a slight variation in the shape of the cysts in the various hosts, there is no distinct difference in the formation of their walls.

Occurrence of the encysted stage in other invertebrates.

In the field the encysted stage occurs in the Invertebrates listed below. It will be noticed that I have been able to infect experimentally most of the natural hosts.

Class INSECTA. Order COLEOPTERA.

Dytiscus marginalis. Cysts occur under cuticle in posterior abdominal regions. Under experimental conditions cercariae readily encyst. During an experiment one beetle larva moulted completely and an examination of the cast revealed 14 cysts at various parts within the abdominal region. The moult was allowed to remain in water. The encysted larvae were fairly active after two days, but movement ceased at the end of the third day. Thereafter the cysts became turgid and finally decomposed.

Order NEUROPTERA.

Sialis lutarius. Two encysted larvae in one specimen dissected. Cysts oval, larvae active, stylets still *in situ*; infection probably recent. Many specimens have been examined but no more cysts have been met with. Experimentally cercariae do not readily encyst.

Order TRICHOPTERA.

Halesus sp. Cysts present in from 20 to 30 per cent. of specimens examined. Two to six cysts in one host. Easily infected experimentally.

Limnophilus centralis. During June and July 1924, 30–40 per cent. infected. From 10 to 20 cysts present in one host but as many as 50 may occur. Cysts occur throughout the whole abdominal region. Experimental infection easy.

Limnophilus rhombicus. Three hosts examined and three cysts obtained, one from each larva. Experimental infection easy.

Plectrocnemia conspersa. One natural infection obtained. Four cysts present in mid-abdominal region. Experimental infection easy.

Rhyacophila dorsalis. One natural infection obtained. Two oval encysted stages present. Not readily infected experimentally.

Order DIPTERA.

Tipula maxima Poda. On one occasion only, out of many dissected. Seven oval cysts present, all associated with the outer lining of the alimentary canal in the posterior third of the body. Infection experiments have yielded negative results.

Class GASTEROPODA.

Limnaea peregra Müll. On two occasions, and then only when the digestive gland of the mollusc contained sporocysts of the same species. On first

occasion one cyst occurred in the tissues of the liver, and on the second 7 cysts in the liver and 3 near the salivary glands. Attempts at experimental infection have failed.

A number of invertebrates, chiefly insect larvae not previously found to harbour the encysted stage were also used for infection experiments and positive results were obtained with the following.

Order DIPTERA.

Pedicia rivosa. One larva used; five cysts obtained from extreme posterior region after 2 hours' close contact with mature cercariae. Cysts oval and of normal size.

Culex pipiens L. Twenty-five gnat larvae nearing pupation were used. Twenty were examined after 12 hours' contact with cercariae, when 14 were found infected with an average of 3 cysts each. All were located in the thoracic region. The remaining 5 larvae were allowed to pupate out of contact with cercariae. Two pupae were examined but no cysts were found. Of the 3 imagines which emerged two cysts were found in the thoracic region of one and one cyst in the mid-abdominal region of another. The third was uninfected.

Of a different encysted distome occurring naturally in *Culex pipiens* Eckstein (1922) observes that the cysts are present in the last four or five segments of the abdomen, and that the thorax is usually free from cysts. Ruge (1903) has also recorded encysted cercariae from gnats.

Class CRUSTACEA.

Orchestia littorea. This amphipod is as easily infected experimentally as is *Gammarus pulex.*

Class TURBELLARIA.

Polycelis cornuta Schm. Encystment takes place only after prolonged contact with mature cercariae. Not more than 3 cysts have been found in one host.

As a point of interest it might be noted, that under experimental conditions the cercaria is able to encyst in certain terrestrial insect larva, *e.g. Bibio* sp., also in "*Slaters*" centipedes and millipedes, but not in earthworms.

From the above it is evident that the encysted stage has a wide range of hosts. It may be safely said that encystment normally takes place in *Gammarus pulex* and certain caddis-fly larvae. In the literature there are numerous records of larval distomes in *Gammarus pulex* and in insect larvae, but instances of the same larva in both appear to be rare. One case is recorded by Dollfus (1924), who claims that *Gammarus pulex* may act as intermediate host to the encysted stage of *Pleurogenes medians* Olsson, Ssinitzin having previously found the encysted stage in various insect larvae. Dollfus considers encystment in *Gammarus* not as a temporary adaptation, but as an evolutionary one, the encysted individuals continuing to develop normally and leading to a partly functional reproductive system just as in the largest individuals described by Ssinitzin.

Records of encysted larval Trematodes in Turbellarians are also rare. The only papers I have been able to see in this connection were those of Mrazek (1910) and Joyeux (1920). The former describing cysts from an oligochaete, adds that he also saw them in triclads. The distome described by Joyeux encysts naturally in *Dendrocoelum lacteum* Müll., three or four cysts occurring in natural infections, but he obtained more experimentally. Joyeux (*loc. cit.*) also records the following instances of encysted larval Trematodes in Turbellarians: Sekera (1888) in *Microstomum lineare* Müll.; Dorner (1902) in *Derostoma unipunctata* Oerst., and v. Graff (1903).

Identification.

Following Lühe's classification this cercaria comes under the group of *cercariae armatae* and among these may be placed near *C. secunda* in the following part of the key:

11. Bauchsaugnapf $\frac{3}{4}$ des Durchmessers des Mundsaugnapfes nicht erreichend. Bohrstachel 0·029–0·03 mm. lang.
    a. Ösophagus lang, Darmgabelung dicht vor dem Bauchsaugnapf. *C. limnaeae ovatae.*
    b. Ösophagus kurz, Darmgabelung weit vor dem Bauchsaugnapf. *C. secunda.*

Cercaria secunda, however, differs in its characteristic armature of long body bristles.

Of the fresh-water ziphidiocercariae described since 1909 *C. intermedia* from *Limnaea stagnalis* (Wunder, 1923 *c*) and *C. leptosoma* from *Limnaea peregra* (Brown, 1926) are the only European species comparable. *C. intermedia* is as yet only imperfectly described but it differs greatly in the length of the mature sporocysts, in the relative sizes of the suckers and the length of the stylet and tail. *C. leptosoma* differs in the form of its alimentary canal and in the fact that it does not encyst in *Gammarus pulex*.

But attention must be drawn to the remarkable resemblance the encysted stage of *Cercaria* X. 1 bears to *Distomum pulicis*, an advanced larval distome described by v. Linstow (1892) from *Gammarus pulex*. From a comparison of these two I consider *Cercaria* X. 1 a likely early stage of *D. pulicis* v. Linstow. Table V shows the close agreement.

Table V.

D. pulicis v. Linst.	Encysted stage of X. 1
Cyst	
Length: 0·27–0·31 mm.	0·2–0·27 mm.
Breadth: 0·2–0·23 mm.	0·15–0·19 mm.
Stylet: 0·026 mm.	0·026–0·029 mm.
Contents removed	
Length: 0·44 mm.	0·42–0·44 mm.
Breadth: 0·14 mm.	0·12–0·16 mm.
Oral sucker: 0·104 mm.	0·08–0·1 mm.
Ventral sucker: 0·06 mm.	0·04–0·05 mm.
Pharynx: 0·042 × 0·038 mm.	0·03 × 0·25 mm.
Oesophagus: absent	Very short

Brown (1926) has identified the encysted stage of his *Cercaria pulicis* from *Gammarus pulex* with *D. pulicis* v. Linst., but for the following reasons I cannot identify his cercaria with my own:

(1) The sporocysts of *C. pulicis* are 1 mm. long and white, while in *Cercaria* X. 1 they often exceed twice that length and are yellowish-brown in colour.

(2) The oesophagus of *C. pulicis* is long and bifurcates immediately in front of the ventral sucker, whereas in *Cercaria* X. 1 it is short and bifurcates a little behind the pharynx.

(3) The stylet in *C. pulicis* is 0·032 mm. long while in *Cercaria* X. 1 it is 0·026–0·029 mm. long.

In spite of this noteworthy difference in the length of the oesophagus, the encysted stage of *C. pulicis* is in close agreement with my own. Thus the oesophagus of *C. pulicis* must undergo a decrease in length during encystment. Brown gives no measurements in this connection, but I take it the oesophagus of the cercaria is approximately 0·11 mm. long while in the encysted stage it is approximately only 0·023 mm. long. Further, if studied separately, *C. pulicis* and its encysted stage fall into different groups in Lühe's key.

The regions of the intestine, as seen in the encysted stage of *Cercaria* X.1, bear to each other approximately the same relations as in the cercaria and, further, the intestine of the cercaria persists in the encysted stage, there being no breaking down and rebuilding of tissues as seen in some furcocercariae. Brown gives no details of any infection experiments he may have carried out.

Cercaria X. 2. (Fig. 4 *A*, *B*.)

The sporocysts occur in the digestive gland of *Limnaea truncatula* Müll. They are as a rule very numerous and much involved. Mature specimens vary in length from 1 to 3 mm. and in breadth from 0·3 to 0·42 mm. The body is smooth and tapers slightly towards both ends. They are colourless to begin with but as development proceeds they acquire an orange-yellow colour with black pigment spots here and there. Young sporocysts exhibit slight elongations and contractions but all active movement ceases before maturity. From 20 to 30 active cercariae are present in one sporocyst. The germinal area is non-localised and there is no birth pore.

The origin of infection appears to be at a point near the hermaphrodite gland from which it gradually extends through the whole digestive gland. In heavy infections the gonads are also destroyed.

The cercaria is broadly oval in outline and faintly straw-coloured. The body is flattened and of uniform thickness and is armed on its anterior fourth with transverse rows of very minute spines. A stylet 0·023 mm. in total length is present in the roof of the oral sucker. The nib of this organ is 0·004 mm. long, and carries two minute lateral reinforcements. The two suckers are of about equal size, the ventral one being situated near the centre of the body. The tail is slender and about the same length as the body. The caudal pocket is sub-terminal.

Internal anatomy. The mouth leads to a short pre-pharynx and an oval muscular pharynx. The oesophagus bifurcates about 0·03 mm. behind the

pharynx and the intestinal loops do not extend much beyond the anterior margin of the ventral sucker.

The excretory vesicle is bilobed and prominent and both its median stem and lateral lobes are contractile. The excretory pore is dorsal. Owing to the opacity caused by the glandular content of the body, details of the system are difficult to trace. An anterior and a posterior tubule unite on each side of the body, about one-third of the distance from the posterior margin in a fairly wide collecting canal, which, after a looped course enters the lateral lobe of the vesicle. Both anterior and posterior tubules appear to end dichotomously

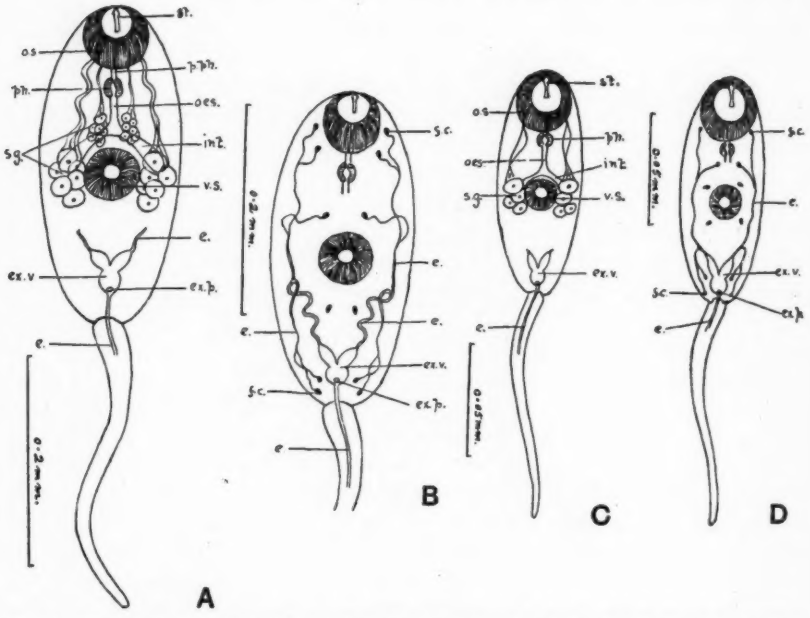

Fig. 4. *Cercaria* X. 2. *A*. Cercaria showing alimentary canal and glands. *B*. Cercaria showing excretory system. (For lettering see Fig. 1.)

Cercaria X. 3. *C*. Cercaria showing glands, etc. *D*. Cercaria showing excretory system. (For lettering see Fig. 1.)

in capillaries with flame cells, while the anterior tubule on each side also receives a tributary from a flame cell situated on the shoulder of the intestinal loop. Other flame cells occur in positions indicated but their capillaries were not traced. A relatively wide median tubule is present in the tail.

Six large stylet gland cells with prominent nuclei lie on either side of the ventral sucker. Normally these cells are round with a diameter of 0·02 mm. but they alter greatly in shape with the degree of contraction of the cercaria. They communicate with the oral sucker by means of two sinuous ducts. Two groups of smaller cells of diameter 0·01 mm. lie near the median line just

anterior to the ventral sucker and dorsal to the alimentary canal. A single duct from each group passes anteriorly to the oral sucker. Cystogenous cells are most abundant in the lateral regions of the posterior two-thirds of the body. Other measurements are given in Table VI.

Table VI.

	mm.
Length	0·28–0·3
Breadth in region of ventral sucker ...	0·14
Oral sucker	0·06
Ventral sucker	0·056
Pharynx	0·026 × 0·021
Excretory vesicle	0·02
Length of tail	0·28

Activity and duration of free life. The mode of escape of the cercariae is probably by rupture of the sporocyst. The cercariae are exceedingly active, the muscular tail executing rapid lashing movements behind and under the ventral surface of the body. They survive for 6 days in pond water, but no swimming was observed after the first 36 hours.

I have been unable to find the encysted stage of this cercaria in the field, and under experimental conditions I have tried *Limnaea peregra*, *Gammarus pulex*, *Dytiscus marginalis* and *Limnophilus rhombicus*, but with negative results.

Identification.

The only European species comparable is the probable early stage of *Distomum homalostomum* (v. Linst., 1877, pp. 104–5). The latter occurs encysted in *Limnaea ovata* Drap. and *Succinea putris* L., while the cercaria develops in *Limnaea stagnalis* L. Apart from noteworthy differences in the length of their mature sporocysts, and the length of the body and stylet of the cercariae in question, it will be recalled that I have been unable to obtain the encysted stage either in the field or experimentally, so that it seems unlikely that the two are identical.

Cercaria Limnaea truncatula (v. Linst., 1892, pp. 331–2) appears to be a close relative, but differs in the size and colour of its sporocysts, and in its smooth cuticle and form of its stylet. Its further life-history is unknown.

Cercaria X. 3. (Fig. 4 *C*, *D*.)

The sporocysts which occur in the digestive gland of *Valvata piscinalis* Müll. are small, plump and of simple structure, the epithelium being very delicate. The majority are almost as broad as long, the average measurements being 0·25 × 0·22 mm. They are colourless and exhibit no active movement. The cercariae are proliferated from a non-localised germinal epithelium lining the whole cavity. From 6 to 10 active cercariae may occur in a sporocyst at one time. No birth pore was observed. The sporocysts are numerous and very involved, considerable fusion of their walls taking place.

The cercaria is oval in outline and exceedingly minute, the length being only about 0·09 mm. and the maximum breadth 0·035 mm. Its anterior end is armed with minute spines arranged in transverse rows which dwindle posteriorly and finally disappear about 0·03 mm. from the anterior margin. The oral sucker is relatively large and armed in its roof with a simple stylet 0·018–0·02 mm. in length. The shaft of the stylet is approximately 0·011 mm. and is narrowest in the middle. At the base of the nib is a collar-like rim. The ventral sucker is smaller than the oral sucker and is situated a little behind the centre of the body. The cylindrical tail is slender and muscular; it is slightly longer than the body and inserted terminally.

Internal anatomy. The oral sucker is followed by a short pre-pharynx and a small pharynx. The oesophagus bifurcates about 0·03 mm. behind the pharynx, but owing to the opacity produced by the gland masses the entire length of the intestinal loops could not be followed.

The excretory vesicle consists of a small body with two cornua each of which receives a single anterior and a single posterior tubule. The anterior tubule is the more prominent of the two and often contains numerous minute excretory granules. Both anterior and posterior tubules end in two capillaries with flame cells, while two other flame cells occur on either side in the positions indicated in the sketch. In the tail, the system is represented by a narrow median tubule. The excretory pore is dorsal.

Four relatively large gland cells with yellowish brown granular contents and prominent nuclei are arranged on either side of the ventral sucker. The two anterior cells of each mass are slightly larger than the posterior two. A single duct passes anteriorly from each glandular mass, it dilates about the level of the pharynx but narrows again before opening into the roof of the oral cavity.

Additional measurements are given in Table VII.

Table VII.

	mm.
Length	0·08–0·1
Breadth in centre of body	0·05
Oral sucker	0·028
Ventral sucker	0·02
Pharynx	0·015
Length of tail	0·11

Activity and duration of free life. The cercariae swim head first by lashing movements of the tail which is not easily shed. In the process the body is much contracted and appears almost globular. The periods of activity are long, from one to two minutes, and the progress is fairly rapid. They survive from 30 to 40 hours in pond water.

Identification.

This cercaria belongs to a group of very small forms called *Cercariae microcotylae* (Lühe, 1909, pp. 196–8) the chief characteristics of which are as follows:

(*a*) Body less than 0·2 mm. long.

(*b*) Ventral sucker appreciably smaller than the oral sucker and behind the middle of the body.

(*c*) Stylet glands 2–4 in number, and often yellowish, brownish or greenish in colour.

Of these *Cercaria microcotyla* Fil. from *Viviparus viviparus* L. is a close relative, but differs in the size and shape of its stylet.

At present no suggestion can be made as regards the further life-history of *Cercaria* X. 3. Looss (1896) suggests that certain xiphidiocercariae of this type, described by him, may be related to small distomes found in the intestines of chameleons and lizards in Egypt.

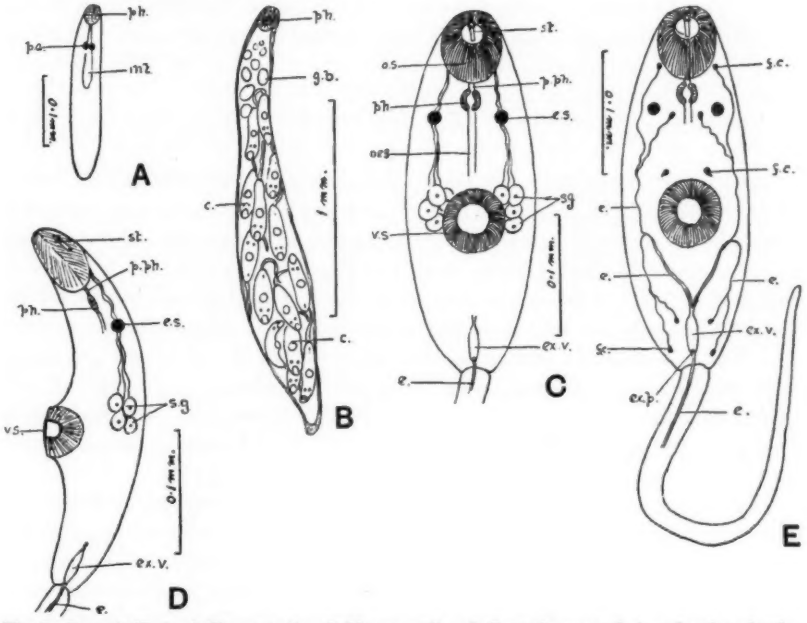

Fig. 5. *Cercaria* X. 4. *A*. Young redia. *B*. Mature redia. *C*. Cercaria, ventral view showing glands, etc. *D*. Cercaria, lateral view. *E*. Cercaria showing excretory system. (For lettering see Fig. 1.)

Cercaria X. 4. (Fig. 5 *A–E*.)

The rediae occur in the small bivalve, *Pisidium personatum* Malm., and are light brown in colour and of irregular contour. They are located in the digestive gland of the host and are not numerous, as a rule only from 6 to 10 occurring in one host. The length is from 1·8 to 2·2 mm. The pharynx is strongly muscular and is protrusible. Young rediae are straight, colourless and of almost uniform thickness, while a small black pigment area is present a short distance behind the pharynx. This pigment area consists of two closely apposed spherical masses of pigment connected by fine pigment granules, each mass being approximately 0·01 mm. in diameter. The pigment gradually

disappears as the redia grows, and was not traced in larvae more than 0·6 mm. long. As growth proceeds the rediae acquire the power of slowly oscillating their anterior ends, but the movement is never pronounced and in the adult is practically limited to the protrusion and withdrawal of the pharynx.

Internal anatomy. A thin-walled sacculate intestine reaching a little below the level of the pigment area was observed in most specimens less than 0·3 mm. in length. The intestine disappears long before the redia is full grown and is probably never functional. The wall is 0·021 mm. in thickness about the middle of the body increasing to 0·056 at the poles. Beneath the cuticle is a layer of thick-walled cubical cells with granular contents, and internal to this a layer of flattened epithelial cells. The area of cell proliferation is in the anterior end of the redia, the cercaria being pushed posteriorly as they develop. From 10 to 20 active cercariae may occur in one redia. No birth pore was observed. For further measurements see Table VIII.

The cercaria is colourless, transparent, oval in contour and very contractile.

The length is 0·3 mm. and the maximum breadth 0·1 mm. in the region of the ventral sucker. There are two suckers of almost equal size. The oral sucker is globular and sub-terminal and armed with a prominent stylet. The total length of the latter varies from 0·028 to 0·03 mm. Its nib is approximately 0·006 mm. long and is reinforced by a ventral thickening round its base. The cup-shaped ventral sucker is situated about the middle of the body and almost constantly protrudes. Its outer margin is thickened and beset with minute tubercles arranged in two rows. At maturity the margin of this sucker assumes a light brown colour. The tail is inserted sub-terminally and is slender and slightly longer than the body. Conspicuous on the anterior-dorsal aspect of the cercaria are two black-pigment eye-spots.

Internal anatomy. The mouth leads through a short prepharynx to a small pharynx. Then follows a long oesophagus reaching almost to the ventral sucker. The bifurcation of the oesophagus was not observed.

The excretory system consists in the main of a muscular vesicle elongated in the middle line, a short narrow collecting canal directly anterior to the vesicle, and two main excretory tubules on each side of the body. One tubule is directed posteriorly while the other runs forward lateral to the ventral sucker, and in the head region forms two branches the lower one of which ends in a flame cell over the oral sucker. The posterior tubule also ends dichotomously in flame cells. Two other flame cells were seen on either side but their connection with the system was not traced. In the tail is a single median tubule. The excretory pore is dorsal at the posterior end of the vesicle.

Anterior to the ventral sucker are eight stylet gland cells divided into two adjacent groups of four cells each. These cells are rounded with a diameter of 0·014 mm. and their contents are coarsely granular. A sinuous duct connects each group with the oral sucker. For other measurements see Table VIII.

Table VIII.

	mm.
Redia	
Length	1·8–2·2
Breadth	0·28
Pharynx	0·04
Cercaria	
Oral sucker	0·06
Ventral sucker	0·056
Ventral sucker may protrude	0·04
Pharynx	0·02
Length of excretory vesicle	0·03
Diameter of eye-spots	0·015
Distance apart of eye-spots	0·06
Distance of eye-spots from anterior margin	0·08
Length of tail	0·36

Activity and duration of free life. The cercariae swim rapidly, the tail executing rapid lashing movements under the body. While in suspension in water they often bend themselves into a U shape with the ventral sucker protruding markedly inwards. They are positively phototropic. In tap water they survive from 24 to 30 hours.

Identification.

There appears to be no cercaria in the literature comparable with the above. Its body bears some resemblance to that of *C. isopori* Lss, but the tail of the latter is as broad, or broader, than the body and carries two marginal cuticular projections.

VI. SUMMARY AND CONCLUSIONS.

A large number of fresh-water Invertebrates has been examined and the parasites obtained from them include examples of the commoner groups to be met with in any area surveyed. Six larval Trematodes have been described in detail, and by means of direct animal experiment two of them have been definitely related to known adults. Of these two one is the larva of *Notocotylus seineti* Fuhr., and is doubtfully *Cercaria monostomi* v. Linst., while the other is the larva of *Echinoparyphium recurvatum* v. Linst., and is described here in detail for the first time. The four xiphidiocercariae described are new. Data relating to the activity, duration of free life, and process of encystment of cercariae within the bodies of secondary intermediate hosts is also given.

Effect of the parasite on the host. The disruption of the tissues brought about by the presence of sporocysts or rediae in the liver of a mollusc injures the host, and in many cases brings about its death. The gonads also are often attacked and completely destroyed. This latter feature of parasitic castration has been observed by Giard (1888) among certain species of *Limnaea*, *Planorbis* and *Paludina* attacked by sporocysts. Brown (1926) states that the gonads are rarely infected. As regards the encysted stages of cercariae these appear to do very little harm to their hosts even when present in large numbers. These observations are in agreement with those of Lebour (1912) for the sporocyst, rediae, and encysted stages of marine Trematodes.

Double infection. I have found no instance of a double infection among the species described. In the literature, several records show the parasitism of two or more species of cercariae, within one and the same host. The occurrence of two species is most commonly met with, and as a rule one is in predominance. Sewell (1922) states that double infection is not common in India. Faust (1917), on the other hand, has found as many as four trematode species within the same host individual (*Planorbis trivolis*). Hesse (1923) states that "every large *Limnaea peregra* examined was heavily infected with sporocysts of either of the two species, but it was rare to find both together." Brown (1926) in the neighbourhood of Birmingham has found three examples of associated xiphidiocercariae and furcocercariae, one example of xiphidiocercariae and echinostome cercariae, and two examples of echinostome cercaria, all from *Limnaea stagnalis* L.

VII. ACKNOWLEDGEMENTS.

For counsel and encouragement during the investigation I desire to acknowledge indebtedness to the late Prof. Gemmill, F.R.S. My best thanks are also due to the following authorities through whose kindness the invertebrate hosts were named:

Molluscs: by Mr H. Coates, F.S.A. (Scot.), of Perth, who in some cases had the specimens examined and authenticated for me by the Recorder and Referees of the Conchological Society. For the sake of uniformity these authorities have adhered to the classification and nomenclature adopted in Roebuck's Census of June 1921 as being the latest authoritative system.

Crustaceans: by Dr W. T. Calman, F.R.S. Insect larvae by Messrs F. Edwards and K. Blair, all of the British Museum.

The adult parasites obtained from infection experiments were named by Dr H. A. Baylis also of the British Museum (Natural History). I have also to thank Prof. A. D. Peacock, D.Sc., F.R.S.E., for advice during the preparation of this paper.

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THE LIFE-HISTORY OF A MARINE TREMATODE, *HAMACREADIUM MUTABILE* LINTON, 1910[1].

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(With 3 Text-figures.)

During the summer of 1928 the writer carried out life-history experiments upon a number of the cercariae occurring in marine molluscs at Tortugas, Florida. One of the cercariae studied, a stumpy-tailed larva belonging to the Cotylocercous group, was found to encyst in the muscles of small fish. Feeding experiments were carried out with fish experimentally infested with the cysts, and adults were developed in the gray snapper, *Neomaenis griseus*. These worms were identified as *Hamacreadium mutabile* Linton, 1910, a member of the sub-family Allocreadiinae. A brief preliminary report of the observations in this paper has already been published (McCoy, 1928).

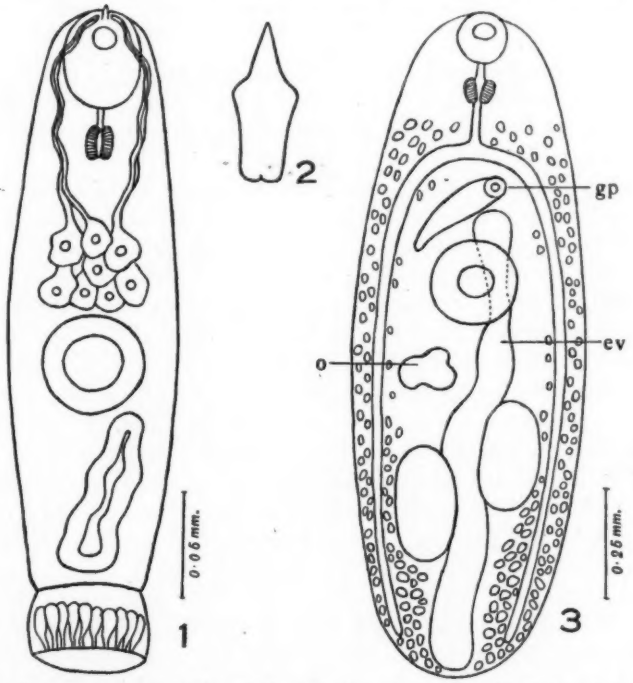
The cercaria of *Hamacreadium mutabile* occurs in *Astraea americana*, and was first found at Tortugas by Miller (1925 *b*) who described it as Cercaria A in his preliminary report of a survey of Tortugas cercariae. Since his description includes only a brief account of the essential features, further details will be given in this paper.

The cercaria of *H. mutabile* (Fig. 1) has a mobile elongate body which is characterised by the possession of a very short cup-shaped tail. The tail, which is only about one-eighth the length of the body, is not used in locomotion but as a sucker; the cercaria attaches itself to the substratum with it and then waves the body back and forth through the water. There are a number of glandular cells in the tail which open individually on the posterior surface. The body of the cercaria is very extensile and has a thick cuticula which is devoid of spines. Measurements of the cercaria are included in Table I (p. 222) in comparison with those of more mature stages in the life-cycle.

The cercaria of *H. mutabile* belongs to the Cotylocercous group, a well-defined marine group separated from the microcercous cercariae by Dollfus (1914). These cercariae are characterised chiefly by the short, cup-shaped tail, the possession of a stylet and stylet glands, and a thick-walled, non-bifurcated excretory vesicle; development is in simple elongate sporocysts. The cercaria

[1] The work was done at the Carnegie Marine Biological Station at Tortugas, Florida. The writer is indebted to Dr W. W. Cort for aid in the preparation of the manuscript.

of *H. mutabile* possesses all of the typical characters of the Cotylocercous group. A small stylet 10μ long with a single point (Fig. 2) is present on the dorsal lip of the mouth. The central part of the body anterior to the ventral sucker is occupied by eight conspicuous granular glands the nuclei of which could easily be counted under low power magnification. Narrow inconspicuous ducts from these glands pass forward in two bundles to empty at each side of the stylet. A well-developed pharynx is present immediately



Figs. 1–3. *Hamacreadium mutabile.* (1) Ventral view of cercaria. (2) Stylet of the cercaria. (3) Ventral view of specimen from intestine of gray snapper 17 days after infestation. Abbreviations: *ev*, excretory vesicle; *gp*, genital pore; *o*, ovary.

posterior to the oral sucker but the remainder of the digestive tract was not distinguished. The ventral sucker, which is located posterior to the centre of the body, is slightly larger than the oral sucker. The excretory vesicle is large, thick-walled and non-bifurcated, and occupies the posterior part of the body. Its anterior end lies to the left of the ventral sucker. The walls are composed of large granular cells and are so thick that when collapsed, the vesicle appears as a solid structure. The flame cell pattern was not determined.

The cercariae developed in large, simple, elongate sporocysts ranging up to several millimetres in length. The body-wall of the sporocyst contained masses of bright yellow-orange pigment. In mature infestations, the gonads

Table I.

Size measurements of stages in the life-cycle of Hamacreadium mutabile[1].

	Cercaria	Metacercaria 11 days old	Developing worms. Days in primary host			
			2	8	11	17
Length of body	0·268	0·396	0·420	0·800	1·113	1·712
Width of body	0·104	0·165	0·187	0·375	0·454	0·562
Oral sucker	0·052	0·063	0·065	0·112	0·144	0·153
Ventral sucker	0·059	0·100	0·092	0·177	0·230	0·258
Pharynx	—	0·028	0·034	0·064	0·076	0·098

of the snails containing the sporocysts appeared orange in colour and the infested snails were easily detected. Only 2·4 per cent. of over a thousand specimens of *Astraea americana* dissected during the course of the summer were found to be infested.

The cercaria of *H. mutabile* was found to encyst in the tissues of small fish exposed to large numbers of the cercariae in a small amount of water. Four species of fish, *Haemulon sciurus*, *Neomaenis synagris*, *Halichoeres bivittatus* and *Sparisoma flavescens*, were used in the experiments and all became infested. The cysts containing the metacercaria stages occurred in the tissues throughout the body but were most numerous at the base of the gills and in the soft tissues around the mouth. The cercariae encysted within a few hours after penetrating the fish forming an oval cyst with a thin, transparent wall. The average size of five cysts was 225μ by 130μ. In metacercariae dissected from the cyst three days after penetration, the digestive tract which was not present in the cercaria had definitely appeared. There was a long oesophagus forking in the anterior third of the body into caeca which extended to the posterior end. The stylet was no longer present but traces of the glands and gland ducts were still visible. The excretory vesicle was filled with concretions and occupied the greater part of the body posterior to the ventral sucker. Metacercariae which had been in the fish for eleven days were appreciably larger than the cercaria (see Table I), but the only morphological changes were the appearance of the digestive tract and the disintegration of the stylet and stylet glands.

Small fish experimentally infested with cysts were fed to three species of larger fish, the common grunt, *Haemulon plumieri*, black grouper, *Mycteroperca bonaci*, and gray snapper, *Neomaenis griseus*. Development of the worms took place only in the gray snapper. Five full-grown gray snappers kept in separate aquaria in the laboratory were used in the feeding experiments, the fish being killed at intervals of from two to seventeen days after infestation. A summary of the feeding experiments is given in Table II. Cysts were infective in as short a time as three days after the penetration of the cercariae; the infectivity of cysts younger than this was not tested. As many as 446 worms, all in the same stage of development, were recovered from the intestine and

[1] All figures are in mm. and are the average measurements of from 5 to 10 specimens killed by gentle heat.

Table II.

Specimens of Hamacreadium mutabile *recovered from experimentally fed gray snappers.*

Fish number	1	2	3	4	5
Age of cysts fed (in days)	3	12	10	15	11
Killed (days after feeding)	2	3	8	11	17
Worms recovered	295	35	446	56	19

pyloric caeca of the most heavily infested fish. Five control gray snappers caught in the same lot with the experimental fish were examined, two of which had natural infestations of *Hamacreadium mutabile*. One of the fish harboured two adult worms, while the other contained only one. The massive infestation of the experimentally fed fish and the fact that a graded series of developmental stages was obtained by killing the fish at different intervals after feeding is considered to be conclusive evidence establishing the life-cycle.

Although no natural occurrences of the metacercaria were found in the examination of numbers of small fish from the reef where the infested snails were collected, it is most probable that the experimental life-history with encystment taking place in small fish is the one that occurs in nature. The gray snapper is a predatory fish and feeds naturally upon smaller fish and crustacea. The cercariae failed to encyst in small crabs and amphipods which were tested as other possible second intermediate hosts.

The worms grew rapidly after their introduction into the primary host. The increase in size is shown by the measurements of the various stages listed in Table I. After eight days the worms had nearly doubled in size and the testes and cirrus pouch had started to develop. Also, the excretory vesicle had greatly elongated and contained numerous small concretions. In living specimens it could easily be distinguished as a dark structure extending from the posterior end of the body to a region slightly anterior to the ventral sucker. By the end of 17 days the worms had nearly reached the adult size but were not quite sexually mature (Fig. 3, p. 221). The male reproductive system was fully developed, for the cirrus pouch was completely formed and motile sperm were present in the seminal vesicle. Parts of the female reproductive system had also appeared. Vitellaria were present along the sides of the body posterior to the pharynx and the ovary (*o*, Fig. 3) could be distinguished anterior to the right testis, but no ova were yet present in the uterus. The laboratory closed before there was time to obtain sexually mature specimens experimentally, but the worms which had been developing in the fish for 17 days could be identified with certainty as *Hamacreadium mutabile* Linton, 1910. The most important specific characters used in the identification were the location of the genital pore (*gp*, Fig. 3) to the left of the median line and the unusual length of the excretory vesicle (*ev*, Fig. 3).

Linton (1910) originally described *H. mutabile* from the gray snapper at Tortugas and designated it as the type of a new genus belonging to the

allocreadine group of Trematodes. There are at least a dozen genera which have been assigned to the sub-family Allocreadiinae (Poche, 1925), and of these there are seven genera which occur exclusively in marine fish. To the writer's best knowledge the only life-history in the entire group which heretofore has been known is that of *Allocreadium isoporum* Looss (Looss, 1894). The cercaria which is assigned to this species is similar to the cercaria of *Hamacreadium mutabile* in general bodily structure and in the presence of a stylet, but differs in the possession of eye-spots and a large powerful tail. Also, the cercaria of *A. isoporum* develops in rediae whereas the cercaria of *H. mutabile* develops in sporocysts.

The Cotylocercous group of cercaria as proposed by Dollfus (1914) comprised several European marine stumpy-tailed cercariae. Sewell (1922) revised the group into the Linearis group excluding the forms possessing a completely developed digestive tract and adding a fresh-water species, *Cercaria indicae* XXXVIII. Since then, Miller (1925 *a*) has described a new marine species *Cercaria searlesiae* from Puget Sound, and (1925 *b*) reported at least three new marine species from Tortugas. The only previous clue as to the adult relationships of this well-defined group of cercariae has been the knowledge that *Cercaria micrura* Fil. is the larva of *Sphaerostoma bramae* (Lühe, 1909). *C. micrura* is a fresh-water form possessing all of the typical structures of a cotylocercous cercaria but differs from most other species in the possession of a well-developed digestive tract. The adult, *Sphaerostoma bramae*, which is parasitic in the intestine of fresh-water fish, according to Poche (1925) belongs in a separate sub-family under the Allocreadiidae and would not be closely related to the genus *Hamacreadium* which belongs in the sub-family Allocreadiinae.

There is incomplete evidence that another Cotylocercous cercaria found in *Astraea americana* at Tortugas is the larva of a second species of *Hamacreadium*, *H. gulella* Linton, 1910, which is also parasitic in the gray snapper. This cercaria, which is very similar to the cercaria of *H. mutabile* except that it contains 14 stylet glands instead of 8, was also found to encyst in small fish. A preliminary feeding experiment indicates that this species also develops in the gray snapper but the worms recovered were too immature to be identified with certainty. It is probable that other cotylocercous cercariae will be shown to be the larvae of members of the marine genera belonging to the Allocreadiinae. With the further elucidation of the life-histories, it may be possible to make a natural division of the sub-family.

Summary.

A cotylocercous cercaria occurring in the marine snail, *Astraea americana*, at Tortugas, Florida, was found to encyst in small fish as second intermediate hosts.

Fish experimentally infested with the cysts were fed to the gray snapper, *Neomaenis griseus*, and adult worms developed in the intestine and pyloric

caeca which were identified as *Hamacreadium mutabile* Linton, 1910, a member of the sub-family Allocreadiinae.

A general relationship of the cotylocercous cercariae to the family Allocreadiidae is discussed.

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